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BIOLOGICAL BULLETIN

PHYSIOLOGY OF THE ORTHOPTERA.

Hydrogen Ion Concentration of the Blood and Alimentary Tract of Certain Orthoptera (Grasshoppers).

JOSEPH HALL BODINE,

Zoölogical Laboratory, University of Pennsylvania.

Recent investigations on the hydrogen ion concentration of blood and body fluids have dealt almost exclusively with higher vertebrates and particularly with mammals. Little information seems to exist for lower forms, especially the insects. Crozier (1) in recent work with transparent aquatic insect larvæ has strikingly shown the hydrogen ion concentration of different parts of the alimentary canal, while Bishop (2) has pointed out the hydrogen ion concentration of blood in the larva and pupa of the honey bee. Jameson and Atkens (3) have studied the hydrogen ion concentration of the blood and alimentary canal of the silkworm. Since no such information seems to exist for grasshoppers it was thought desirable to study the blood and alimentary canal of these forms. The results herein presented are based upon large numbers of animals comprising seventeen different species.

The method employed in determining the hydrogen ion concentration (pH) was a colorimetric one, essentially similar to that described by Felton (4). Various dyes of the Clark and Lub (5) series were used and in all cases different dyes were employed in checking results so that possible errors due to the color of the blood or body fluids could be eliminated. Blood was collected from the body cavity under oil in capillary pipettes as well as directly from an incision in the body wall. No marked differences in pH values of blood collected with and without exposure to air were noted when determinations were carried out within one minute. The alimentary canal was usually taken out

Species.	Blood.	Fluid from mouth.	Crop.	Cæca.	Stomach.	Int. and Rectum.
Melanoplus differentialis	6.81 (6.4–7.0)	5.60 (5.2–5.8)	5.81 (5.5–5.9)	6.06 (5.8–6.9)	6.52 (5.6–7.5)	6.43 (5.4–7.4)
Melanoplus f. rubrum	6.79 (6.7–6.86)	5.1 (4.9–5.3)	5.37 (5.1–5.6)	6.23 (5.8–6.9)	6.55 (5.9–7.3)	6.20 (5.6–7.3)
Melanoplus bivittatus	6.35 (6.0–6.7)	5.45 (5.4–5.6)	5.60 (5.3–5.8)	5.98 (5.8–6.2)	6.40 (5.8–7.2)	6.58 (5.7–7.5)
Melanoplus scudderi	6.80 (6.6–6.9)	.....	5.43 (5.0–5.9)	6.23 (5.9–6.9)	6.66 (6.1–7.2)	5.86 (5.8–5.9)
Dichromorpha viridis	6.70 (6.4–6.9)	.....	5.62 (5.5–5.8)	5.96 (5.8–6.2)	6.16 (5.8–6.4)	6.28 (5.8–6.6)
Arphia xanthoptera	6.83 (6.7–7.0)	.....	5.6	6.03 (5.9–6.02)	6.46 (6.2–6.6)	6.40 (6.0–6.8)
Encoptolophus sordidus	6.77 (6.7–6.9)	5.75 (5.7–5.8)	5.72 (5.6–5.8)	6.25 (5.9–6.4)	6.58 (5.9–7.3)	6.32 (5.8–7.3)
Dissosteira carolina	6.85 (6.8–6.9)	5.40	5.75 (5.6–5.8)	6.05 (6.0–6.1)	6.30 (6.2–6.4)	5.95 (5.8–6.1)
Trimerotropis maritima	6.80	5.80	5.50	6.00	5.90	6.00
Scudderia furcata	6.70	.....	6.00	5.90	6.10	7.30
Conocephalus ensiger	6.70	.....	6.00	6.00	6.10	7.40
Microcentrum retinervis (?)	6.30	.....	6.00	6.10	6.30	6.40
Shistocerca americana	6.60	.....	5.40	5.75	5.80	5.75
Orphula pelidina	6.75	.....	5.65	6.75	6.50	5.75
Spharagemon bolli	6.80	.....	5.40	6.00	6.00	5.80
Chortophaga viridifasciata	6.80	5.30	6.00	6.90	6.95	6.80
Chortophaga australior	6.80	5.35	6.10	6.90	7.00	6.50

of the animals entire; washed and then split lengthwise so that food particles could be removed. Sections as well as the entire alimentary canal were then tested with the various indicator solutions. When the entire alimentary canal is left in the indicator solution for a short time and then examined under a binocular microscope with strong illumination the differences in pH values of the various parts are strikingly brought out. Animals starved for various periods of time, during which the alimentary canal was completely emptied of food, gave pH values similar to those found for normal individuals.

The accompanying table shows the average as well as the range in pH values for the blood and alimentary canal of the species examined. The average pH value for blood of grasshoppers seems to be 6.8. The dark brown fluid usually emitted by the animal when handled is quite acid in reaction. This fluid is doubtless made up of regurgitated substances from the crop as well as salivary secretions. The crop is generally quite acid in reaction while the blind pouches or cæca seem always to be less acid than the crop. The stomach or that part of the alimentary canal into which the cæca open, varies to a considerable extent in its reaction, being at times quite acid and at other times neutral or alkaline. The middle and posterior end of the stomach are usually less acid than the anterior end. The intestine and rectum, that part of the alimentary canal posterior to the point of entrance of the Malpighian tubules, also varies considerably in its reaction. In some species a rather marked alkaline condition is noted while in others both acid and alkaline reactions are found.

The pH of the blood of grasshoppers is of interest inasmuch as it is approximately the same as that found by other authors for different insects, *e.g.*, honey bee (Bishop 2), silkworm (Jameson and Atkens 3). It would be of considerable interest to know if insect blood functions in the transportation of gases, etc., as in the case of mammalian blood. Some phases of this question have been recently discussed by Bishop (2). Muttkowski (6) in studies on the respiration of insects has pointed out that the blood reacts with the oxidation tests for hæmoglobin regardless of the species of insect. These tests (guaiac, P-diamino-benzene, a-naphthol) have been repeated on grasshoppers used in the

present experiments and positive reactions obtained only in the alimentary canal. As a matter of fact no tissue outside of the alimentary canal was found to give a positive reaction. The reaction in the alimentary canal seems to be confined to the cæca and stomach. It thus seems that for grasshoppers' blood other data are quite desirable before definite respiratory functions, similar to those for mammals, can be definitely attributed to it.

Summary.

The blood of seventeen different species of grasshoppers tested by means of appropriate indicators shows an average pH value of 6.8. The average pH values for different parts of the alimentary canal are: fluid emitted from mouth 5.5; crop 5.7; cæca 6.2; stomach 6.4–7.4; intestine and rectum 6.4–7.2. Oxidation tests for hæmoglobin (guiac, P-diamino-benzene, a-naphthol) have been obtained with the alimentary canal and with no tissues outside of the alimentary canal.

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A SUGGESTION AS TO THE EFFECT OF THYROID GLAND SUBSTANCES ON PROTOPLASM IN GENERAL.[1]

ROBERT A. BUDINGTON.

Of the numerous angles from which the physiology of the thyroid gland has been approached, that of its influence during embryological development has been frequently studied. It is quite unnecessary for the purposes of the present paper to review the large literature of the subject; bibliographies are abundant. Briefly, one may recall that the effect of administering thyroid material to children is well known; Gudernatsch ('12), followed by many others, showed the precocious differentiation which follows the feeding of thyroid gland to tadpoles of Amphibia; Kunkel ('18) noted the response of flesh-fly larvæ to thyroid feeding; its influence on other phyla of animals has been investigated, even the Protozoa having been shown to react with an accelerated rate of metabolism, many structural modifications, a faster rate of reproduction, etc., to a thyroid diet.

In all instances of experimental research the reacting substance is, of course, protoplasm. Its condition, highly organized into the various specialized tissues, studied, experimented upon, and interpreted *en masse*—these facts, and our habits of thought, too often lead us into temporary forgetfulness of *protoplasm* as the fundamental and only vital substance in the material being studied.

As intimated above, it would seem that so many animals from such distinct levels of organization have shown clear responses to absorbed thyroid materials that we may easily conclude that something in thyroid composition affects and modifies animal protoplasm perhaps whenever and wherever it occurs. Correlated with this semihypothetical conclusion, one reflects that

[1] From the Department of Zoölogy, Oberlin College. The writer wishes to express appreciation of laboratory accommodations and numerous courtesies shown by the Department of Zoölogy at Columbia University during the latter part of this investigation.

there is another large world of protoplasm identified with the plant kingdom; and the question arises, "Is *all* protoplasm enough alike so that that of plant associations, although of course never normally exposed to thyroid influences, is, nevertheless, susceptible to them?" If so, then the interest which attaches to the physiological effect of this endocrine substance is much increased; if not, then one of the differences in their characteristics, which perhaps was instituted when animal and plant protoplasms began their long evolutionary divergence, is made roughly apparent.

A further question which arises is: "Are animal and plant protoplasms sufficiently alike so that, if both are susceptible to thyroid materials, they both react in the same manner?" That they apparently do to some infections has been fascinatingly brought out by the investigations of Smith ('20) in his scholarly studies into the nature of crown gall in plants, and its close similarity, etiologically, to human cancer. Very numerous instances of similar or comparable responses of plants and animals to the same physical stimuli could be cited.

The fact which has been taken as a point of departure in the present study is the use of thyroid tissues or extracts in hastening differentiation of vertebrate larval cell-masses into adult-like organs, and the provoking of a general precocious metamorphosis. The query, then, is: "Can thyroid gland materials cause precocious differentiation of unspecialized plant tissues?"

Experimental Methods and Results.

The observations here described were made entirely on roots growing from bulbs of *Narcissus*. Naturally, selection of a root must be made with reference to the special tissue structure it may exhibit. In some forms there is a clear-cut separation between the root-cap and its adjacent tissues; in such cases the cap may be cleanly removed from the adjoining tissues, there being no derivational dependence between them. In other roots the cap-cells are continuous with those of the root proper, a "common initial zone" existing between them, from which zone cells bud off into both adjoining areas. It is to this second type, "Type 5" of Haberlandt ('14), that *Narcissus* belongs; as a convenient reminder of the circumstances, a plan of its root tissue arrange-

ment is shown in a diagram of its longitudinal section in Fig. 1. Neither the "common initial zone" nor the root-cap shows any distinct differentiation; they form an essentially parenchymatous mass. In a proximal direction, however, differentiation is soon met, *i.e.*, the cylindrical periblem, and the centrally placed plerome mass, also a cylinder.

The real question, then, which is being applied to the *Narcissus* root is: "Do the specialized tissues of the root proper extend down farther into the tip in such roots as are grown in nutritive media containing thyroid constituents than they do in roots grown in nutrient media alone?"

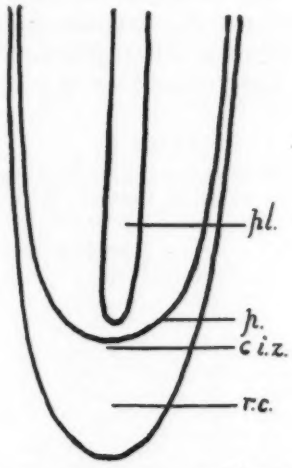

FIG. 1. Showing diagrammatically the arrangement of tissues in the root-tip of *Narcissus;* longitudinal section. *c.i.z.*, common initial zone; *p.*, periblem; *pl.*, plerome; *r.c.*, root cap.

Several dozens of *Narcissus* bulbs were rooted in bottles containing 120 cc. of Pfeffer's nutrient solution to which had been added gland substance (Parke Davis and Co.'s dry thyroid tablets) in these amounts: 2.5. grains, 5 grains, 7.5 grains, and 10 grains, respectively. It is well known, of course, that bulbs differ much in their "strength," and that in consequence of variation in vitality their root growth is not uniform in rate, length and other features; but there is an easily observable average which characterizes the normal or any experimental

line. So far as bulk effect of thyroid substances on root growth in *Narcissus* goes, the result is the same as in *Allium*, reported by the writer in '19; in general, when the control bulbs showed a root length of 60 mm., the thyroid-"fed" line showed a length of 25 mm. These roots were then cut, fixed in aceto-alcohol, stained *in toto* in Delafield's hæmatoxylin, and embedded. Measurements of any region could be most accurately determined by a study of serial cross-sections; these were made 15 micra in thickness.

By count of sections, two measurements were made: (a) the interval from the tip of the root cap to the beginning of the periblem zone; (b) the interval from the tip of the root cap to the central plerome cylinder. To illustrate the uniformity of the result of such measurements, the following table is given, compiled from a series of thirty roots taken at random from among hundreds:

TABLE I.

MEASUREMENTS COMPUTED FROM SECTIONS, 15 MICRA IN THICKNESS, OF *Narcissus* ROOTS GROWING IN 120 CC. PFEFFER'S SOLUTION TO WHICH HAD BEEN ADDED THE EQUIVALENT OF 10 GRAINS OF THYROID GLAND.

	Micra from Tip of Root to Periblem.	Micra from Tip of Root to Plerome.
1	360	405
2	360	420
3	375	420
4	345	405
5	360	420
6	330	405
7	345	405
8	360	435
9	405	465
10	390	480
11	300	360
12	330	390
13	420	495
14	390	435
15	390	450
16	330	400
17	405	465
18	360	405
19	330	390
20	405	450
21	330	390
22	375	420
23	375	420
24	390	465
25	360	405
26	390	465
27	360	420
28	390	465
29	390	465
30	300	390

In the familiar experiments with frog tadpoles, the thyroid-fed line and the control line are, of course, taken from the same batch of eggs, thus being of the same nature and the same age. Similarly, in this case, the bulbs were sprouted in the same nutrient medium, for the same length of time, the sole difference between the experimental and the control bulbs being that the former grew with a given amount of thyroid material added to the solution.

For comparison with the above table, measurements similarly made of the control line are here added:

TABLE II.

MEASUREMENTS COMPUTED FROM SECTIONS, 15 MICRA IN THICKNESS, OF *Narcissus* ROOTS GROWING IN PFEFFER'S SOLUTION ALONE.

	Micra from Tip of Root to Periblem.	Micra from Tip of Root to Plerome.
1	450	555
2	465	550
3	540	630
4	480	540
5	420	480
6	450	495
7	480	555
8	450	525
9	465	540
10	480	540
11	465	525
12	480	540
13	435	495
14	465	510
15	420	495
16	435	495
17	450	510
18	495	555
19	450	510
20	435	495
21	495	570
22	420	465
23	495	570
24	480	540
25	495	570
26	495	570
27	495	555
28	405	480
29	450	525
30	495	570

It seems perfectly clear that the differentiated tissue extends nearer to the tip of the root in the thyroid treated line than it does in the roots of the control line: or, in tangible figures, it extends 20 per cent. nearer the tip.

Discussion

The profound and almost semi-mysterious control of metabolism by thyroid constituents, expressing itself mainly in the phenomena of growth, has attracted many workers to an examination of very varied phases of its possible influence. The present study is but one of this sort; and certain aspects of the facts noted may well receive further comment.

As said above, the main point in mind has been to discover whether thyroid components, if absorbed by elementary rapidly growing plant tissues, will cause in them an accelerated differentiation comparable with what has been repeatedly described as a distinct effect of it when used as a food by, grafted into, or introduced as an extract into Amphibian larvæ. A primary awkwardness immediately presents itself in such an attempt, for the plant root here employed shows such limited specialization as compared with the complexity of an incipient animal organ such as an appendage. The periblem and plerome tissues are the only real differentiations from the parenchyma in a young root, and they show little character in their apical extremities, their identification being essentially dependent on position only. They are, however, *bona fide* differentiations, as truly as muscle, bone, or nerve tissue.

Furthermore, it would be folly to claim that the experimental results here reported are unique to, specific for thyroid "feeding"; there may be dozens of organic and inorganic compounds, so far as the writer knows, which could produce similar or even greater effects of the sort noted than do thyroid components. No attempt has been made to discover or compile such a list. Potassium biniodide has often been employed to offset the effect of thyroid deficiency in children. This fact, however, does not at all lessen one's interest in thyroid substances and their normal physiological values throughout the life-time of the possessing organism.

It may also seem that the interpretation given the experimental

results of this study, as indicating precocious specialization, is arbitrary as against other possible judgments. For example, does not a long root of *Narcissus* normally possess a root-cap thicker than that of a short root? To test this point, a series of roots grown in nutrient solution alone were cut when at the same or less length than those of the thyroid "fed" line, the question of relative age being disregarded. On making measurements of these it was found that the short normal root shows an extent of root-cap equal to or even in excess of that of a longer normal root. The question here at issue can be most easily met by offering the following table of averages of root-cap length (tip to periblem and plerome) of thirty roots each, taken from long-normal (control), short-normal, and thyroid "fed" groups:

	Normal of Same Age as "Thyroid-fed."	Normal of Same or Less Length than "Thyroid-fed."	"Thyroid-fed."
Length of root up to beginning of periblem	463 micra	513 micra	372 micra
Length of root up to beginning of plerome	520.5 "	576 "	412.5 "

It is thus plainly apparent that the cap of the experimental line was more deeply invaded by the specialized tissue than were either of the check roots with which there was any reason for comparing it.

One also naturally raises the question whether the results noted, instead of being those of accelerated differentiation, could not as well be described as simple abbreviation or inhibition of root-cap growth. It is possible that such an interpretation would be perfectly valid; at the same time, one should reflect that the effect is one, not on the root-cap alone, but on the entire root. While the foregoing measurements give information as to only one feature, *i.e.*, length of root-cap, other specifications are also modified by the treatment: *e.g.*, its entire length is less than that of the normal; its average diameter is greater at nearly every part of its growth; it shows tendencies to localized swellings and crookedness. Measurements of these other features of the thyroid "fed" line, however, it is impossible to make in any

satisfactory manner; yet, they may all indicate precociousness. Assuredly, one reflects that hastened specialization of tissues in amphibian larvæ, in dipteran larvæ, in protozoa, were all accompanied by under-size; and Gudernatsch's ('15) experiments with a mammal showed the same diminished size in his thyroid fed line.

The further question whether all protoplasm reacts, and reacts similarly to thyroid components can hardly be answered, even tentatively, while the amount of study of plant responses is so small. Also, one can feel only the most limited concern whether any special plant tissue is amenable to such hormones or not; but the more general inquiry as to the qualities of protoplasm as a whole makes a real appeal. So far as the substance of this group of observations goes, the evidence would seem to suggest an affirmative answer.

Summary.

1. Roots growing from bulbs of *Narcissus* into Pfeffer's nutrient solution to which has been added certain amounts of thyroid gland substance show a growth which is markedly abbreviated as compared with that of the control lines. This result is wholly like that when *Allium* is used.

2. Measurements directed to find out the internal conditions correlated with shorter growth indicate that differentiation of the special root tissues extends nearer to the tip of the root in thyroid "fed" lines than it does in the controls.

3. Assuming that the tissues in freshly growing plant roots can be compared to those in larval animals (Amphibia)—both originate from essentially unspecialized masses, although the plant never reaches the complexity of the animal—it would seem that thyroid substances cause precocious differentiation in both.

4. The suggestion is offered that animal and plant protoplasm are sufficiently alike in their general physiology so that they respond similarly to thyroid substances; or, expressed conversely, thyroid substances are influential in essentially the same manner on all protoplasm.

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ON THE OCCURRENCE AND FOOD HABITS OF CTENOPHORES IN NEW JERSEY INLAND COASTAL WATERS.

THURLOW C. NELSON.

(From the Zoölogical Laboratory of Rutgers University and the Department of Biology of the New Jersey Experiment Station.)

I.

Studies of the plankton organisms of New Jersey inland coastal waters (Fig. 1) which have been in progress since March 1919 reveal interesting facts regarding the occurrence and habits of the ctenophores *Mnemiopsis leidyi* Agassiz, *Pleurobrachia brunnea* Mayer, and *Beroë ovata* Chamisso and Eisenhardt. All three of these forms have been found at times in abundance in New Jersey, but again they may be rare or absent. They must, therefore, be considered as transient visitors to these waters. This paper is presented with the hope that the observations herein recorded may aid in the ultimate solution of some of the problems concerned with the distribution of these comparatively little known animals.

The common comb-jelly or sea walnut of the northeastern coast of the United States, *Mnemiopsis leidyi*, was frequently found by Mayer ('12), off Newport, R. I., in great rafts. It was well known in the Woods Hole region until about 1910 when it practically disappeared and has not since been reported there in numbers.[1] Sumner, Osborn, and Cole ('13), p. 579, report this species as varying from scarce to very abundant throughout the Woods Hole region, where it has been recorded by various observers for every month in the year. It was apparently most abundant here in September and in December. These authors note the irregular occurrence of this ctenophore in different years,

[1] Mr. George M. Gray, Curator of the Supply Department at Woods Hole, informs me in a letter of January 28, 1924, that nearly every year one or two specimens are taken in the winter or in spring. I have been informed that numerous *Mnemiopsis* were found here in September 1924 following an extended period of easterly winds.

it being very abundant during some seasons while absent as in 1904 in others. They state that periods of extreme abundance may occur in winter. Sumner, *loc. cit.*, p. 576, records it in such numbers in Buzzards Bay on November 13, 1907, that the parasitic *Edwardsia leidyi* within the ctenophores were very conspicuous as one looked down from the deck of the ship.

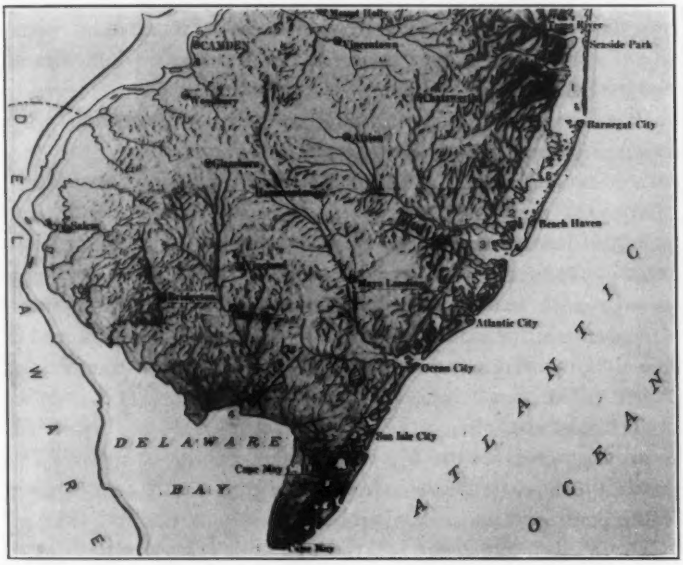

FIG. 1. Relief map of southern New Jersey. 1, Barnegat Bay; 2, Little Egg Harbor; 3, Great Bay; 4, Maurice River Cove. The floating laboratory was stationed from 1919–1920 at Edge Cove, due west of Beach Haven, and from 1921 to the present at Seaside Park.

Bigelow ('15) encountered "myriads" of *Mnemiopsis leidyi* in the surface waters off the New Jersey coast in July, 1913. He found it generally distributed over the inner half of the continental shelf between Barnegat and Delaware Bay, although none was seen north of Barnegat on this voyage.

Pleurobrachia brunnea nov. sp., was found in great numbers by Mayer ('12), on October 16, 1904, off the coast of New Jersey from Barnegat Inlet north to Sandy Hook. The validity of this form as a distinct species has been questioned by Bigelow ('12 and '15), and is discussed in a later section of this paper.

Mayer ('12) lists *Beroë ovata* as abundant along the coast of the United States as far north as Chesapeake Bay. Hargitt ('04) found it common at Woods Hole in 1901, though seldom taken in numbers.

My own records cover only some of the estuaries on the New Jersey coast and do not include the area of oceanic coastal water investigated by Mayer and by Bigelow. During the six years of my investigations *Pleurobrachia* has been found but twice, *Beroë ovata* for short periods during three seasons, while *Mnemiopsis* has been observed daily for months at a time.

Pleurobrachia brunnea was first found by me October 11, 1920, occurring in vast swarms at the surface of water 1–2 meters deep at the mouth of the Mullica River, Great Bay (*cf.* Fig. 1). Associated with it were numerous *Mnemiopsis leidyi* and many small medusæ of several species. So numerous were the *Pleurobrachia* that the water for yards around the boat was white as though with foam. The ctenophores were found in greatest abundance in the tidal slick which forms along the eastern end of the old Graveling natural oyster bed at the mouth of the Mullica River. The first observation of them was at 11 :15 A.M., with bright sunlight, very light NW. wind, tide one third ebb, water temperature 16.6° C., specific gravity 1.0182.[2] The majority of the jellyfish were so close to the surface that they could be dipped up in a fingerbowl for examination.

During the afternoon of the same day large numbers of *Pleurobrachia* were found over much of Great Bay, and on October 15th, 6 *Pleurobrachia* and 3 *Mnemiopsis* were taken in 3 minutes towing with a 15-in. net in Little Egg Harbor. On my next visit to the region, three weeks later (November 3 and 4), no *Pleurobrachia* were found in Great Bay or in Little Egg Harbor. Numerous *Mnemiopsis* were obtained in the former region; the temperature was 13° C., specific gravity 1.0234.

Occasional visits have been made during the autumn in subsequent years but no *Pleurobrachia* have been found. From

[2] All figures for specific gravity are reduced to the basis of distilled water at 4° C. Readings were made with an hydrometer calibrated by the U. S. Bureau of Standards and further checked in some instances by titration of standard sea water obtained from the International Commission for the Investigation of the Sea, Copenhagen. For comparison I have with the aid of Knudsen's Tables transposed Bigelow's salinity figures to specific gravity readings.

all the information I am able to gather this ctenophore appears in New Jersey estuaries for a brief period during October, and then disappears. Mr. Gray informs me that with one possible exception *Pleurobrachia* (supposedly *P. pileus*) was not seen in Woods Hole at all in 1923. That it did not occur in Great Bay during the summer of 1921 I am certain, since our floating laboratory was stationed during this period but a few hundred yards from the place where myriads of these ctenophores were observed in 1920.

My first record for *Beroë* is November 4, 1920, when one half grown specimen was taken in Great Bay; the water temperature was 13° C., specific gravity, 1.0216. No further specimens were seen until October 22, 1921, when 2 large individuals were observed in Little Egg Harbor with temperature of 12.5° C., specific gravity 1.0245. No *Beroë* were found in Little Egg Harbor during the autumn of 1922, nor in Barnegat Bay in the autumn of 1923. A few large specimens were taken in the latter region September 11, 1924, water temperature 16.5° C., and by the close of the month they were abundant. This ctenophore appeared in vast swarms, in the Maurice River, Delaware Bay, about the middle of September 1922 and again at the same time in 1923 and in 1924.[3] It was reported that at times during the flood tide the *Beroë* were so abundant as almost to form continuous rafts. In each of these years the ctenophores appeared about the second week in September while the water temperature was above 20° C. and remained very abundant until early in October when they disappeared as quickly as they had come.

My records of *Mnemiopsis* are much more extensive than in the case of the two preceding ctenophores. The data illustrate in a striking way what other investigators have noted regarding the intermittent appearance of the sea walnut. During the summers from 1908 to 1917 I frequently noted the presence of *Mnemiopsis*, but being occupied with other problems kept no record of its occurrence. From early in 1919 to the present careful records have been kept of the appearance and relative abundance of the ctenophores. Search for these organisms has

[3] I am indebted to two of our former students, Mr. C. A. Perry and Mr. W. H. Dumont, for making these observations and for sending specimens for identification.

TABLE I.

OCCURRENCE OF *Mnemiopsis leidyi* IN NEW JERSEY.

Locality	1919	1920	1921	1922	1923	1924
Little Egg Harbor	Very abundant May to November, 2 qts. obtained in 20 min. tide flow with 15 in. net.	Very scarce during summer. Three taken in 5 min. tide flow on Nov. 4th, temp. 10.6° C. None Nov. 19, temp. 6° C.	None in net in 2 hrs. tide flow May and September. Two caught in a Urosalpinx trap.	None found.	None May 7th; Fairly abundant in late August, remainder of season not known.	Few seen July 24–25. Remainder of season not known.
Great Bay	Not known.	Scarce during summer. Numerous on Oct. 11th with Pleurobrachia. Eight taken in 2 min. tide flow November 3.	None found.	None found.	Occasional specimen seen in late August, Remainder of season not known.	Numerous July 24th. Remainder of season not known.
Barnegat Bay	Not known.	Not known.	Scarce. One specimen 10 cm. long taken in lower Bay Aug. 31. Hit 1 about every 300 ft. at night with launch.	None found. Hit 1 occasionally at night.	Appeared suddenly June 21, specimens of medium size. Increased rapidly and occurred in swarms throughout summer and fall. Very abundant in December, scarce in January, disappeared during February.	Six specimens seen July 3. Great swarms July 8–18.

been made by nets, by traps, and, perhaps best of all where they are scarce, by running a motor boat at night and counting the number of individuals flashing in the wake of the boat. Much of the region covered by these observations is so shallow that the disturbance from a launch's propellor reaches quite to the bottom.

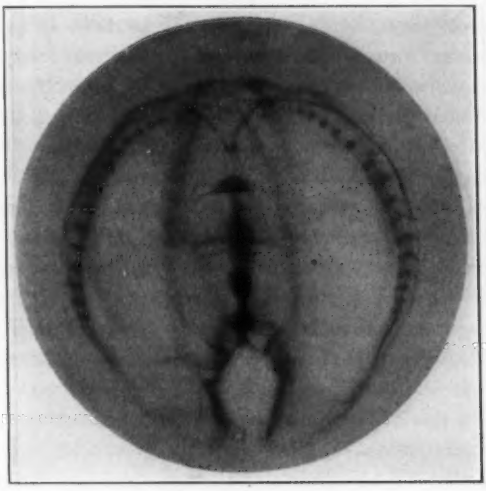

FIG. 2. Young *Mnemiopsis* 5 mm. long in the *Pleurobrachia* stage showing retracted tentacles.

In Table I. a summary is given of our observations of *Mnemiopsis* for the past six years.

Barnegat Bay (*cf.* Nelson, '23*A*) is a shallow estuary with an average tidal fluctuation of 4–6 inches. It therefore warms up rapidly in the spring and presents during the summer subtropical temperature conditions. The temperature of the water on June 21, the date of the first appearance of *Mnemiopsis* here in 1923, ranged from 24.5° to 26.1° C. at 7 stations. The specific gravity varied between 1.0117 and 1.0152. The ctenophores which swarmed in the waters of the bay during July, August and September were mostly of medium or of large size.

As the temperature fell with early autumn particular attention was paid to the stage of development of the animals. All specimens taken October 6, at a temperature of 14° C., were

medium or large. On my next visit, October 20, temperature 15° C., I found several great rafts of *Mnemiopsis* about two thirds of the individuals being approximately 5 mm. long. Among these there were both *Pleurobrachia* and *Bolinopsis* stages of development, bearing strong tentacles (Fig. 2). The smallest were not more than a millimeter long. As indicating the abundance of the animals, one 450 cc. fingerbowl dipped up full of the surface water contained in all 36 young *Mnemiopsis* of which 24 were in the *Pleurobrachia* stage and 12 in the *Bolinopsis* stage. The majority of specimens found on subsequent visits during November, December and January were in the *Pleurobrachia* stage, relatively few large specimens being taken.

In the summer of 1924 the first specimens, 6 of medium size, were found July 3, water temperatures 21.5–22.7° C. Few were observed after this until the 8th when, with temperatures of 24.5 to 24.9° C, the water was literally alive with *Mnemiopsis*. Most of these were newly hatched embryos corresponding to Mayer's Fig. 35 (Mayer, '12, Pl. 6) which represents an embryo of about 30 hours.

Following this heavy spawning of July 6–8 the water swarmed with the ctenophores, numbers as high as 100 per cubic meter being recorded. The animals continued abundant for about 10 days after which they diminished rapidly in numbers, and at no time after this did they approximate the swarms seen in 1923.

The lower limit of temperature at which *M. leidyi* will breed must be very close to freezing, as the following observations show. The fall of 1923 and January 1924 were very mild; our self-registering water thermograph on the Maurice River, Delaware Bay, rarely fell below 5° C. until late in December. The first freeze in Barnegat Bay occurred on the night of January 5th, the bay being covered with ice for about three days. On the morning of January 5, with a temperature of 2° C., numerous medium and small Mnemiopsis were procured.[4]

On my next visit to the region, January 19, no specimens were taken in repeated hauls in the open bay. Medium and very small individuals were fairly abundant, however, in two artificial harbors about 2 meters deep which communicate with the bay by

[4] I am indebted to Mr. Wible of the Physiology Department of Rutgers University for this observation.

narrow outlets. Although these harbors had been frozen over from the 5th of the month until about a week before my visit, the quietness and depth of the water had apparently provided conditions more favorable to survival of the ctenophores than obtained in the open bay. In horizontal hauls of 8 meters each with a net 1 meter square, as high as 19 *Mnemiopsis* were procured per haul. Of these approximately 90 per cent. were in the *Pleurobrachia* and *Bolinopsis* stages and must have come from eggs liberated since the cold weather early in the month.

No further visits were made to the region until March, since the harbors and much of the bay were ice bound from late January until the close of February. On March 8, 1924, water temperature, 3.5° C., no *Mnemiopsis* were found. The adverse conditions resulting from the heavy freezing had evidently destroyed the few survivors in the harbors where they were found on January 19th. The disappearance of *Mnemiopsis* from Little Egg Harbor in November and from the open waters of Barnegat Bay early in January is believed to be due chiefly to the effects of heavy storms which raise much sand and debris from the bottom.

III.

The Behavior of *Mnemiopsis* at Low Temperatures.

Parker '05 found while working with *M. leidyi* during the summer at Woods Hole, that partial cessation of the paddle plates occurred by chilling to 8.5° C., with complete loss of movement at 5° C. The absence of movement at this temperature was shown to be due to causes other than the failure of nervous transmission. If 5° C. represented the temperature below which, in their natural environment, movement of the paddle plates of *Mnemiopsis* ceased, the organisms would perish before late autumn in New Jersey. The temperature of 5° C. as determined by Parker must represent a minimum only for *Mnemiopsis* adjusted to summer temperatures.

At sunset November 9 with the water temperature of Barnegat Bay at 7.5° C., large numbers of *Mnemiopsis* of all sizes were found wherever sought. The following night was cold with a sharp north wind. At 8 :30 the next morning numerous *Mnemiopsis* were seen swimming actively at the surface near the

floating laboratory in water with a temperature of 3° C. Several animals were at once dipped up to make certain that the swimming plates were actually in motion.

To determine the minimum temperature at which the paddle plates would beat in winter, on January 19, 1924, I took *Mnemiopsis* from the water beside the laboratory at a temperature of 4° C. With the aid of a pack of ice and salt the water containing the animals was cooled down until at—0.7° C. water and ctenophores became a mass of ice.[5] As the temperature fell the paddle plates continued beating without interruption. Ice crystals formed about the ctenophores, finally enclosing them, yet while half of an animal was solidly embedded in the advancing ice the paddle plates of the free half continued beating as before. Not until the impinging ice crystals actually imprisoned the plates and held them fast did movement cease.

Mayer ('14) emphasized the fact that whereas tropical marine animals commonly live within 5° C. of their temperature of maximum activity and within 10–15° C. of their upper death temperatures, marine animals of the temperate or arctic regions show but little change in activity within a considerable range of temperatures. Hunter ('04) showed that *Mnemiopsis leidyi* is relatively more resistant to a decrease than to an increase in temperature of the water. The bearing of these observations on the distribution of *Mnemiopsis* in New Jersey will be considered in the last section of this paper.

IV.

The Food Habits of Ctenophores.

Little is known of the feeding and food habits of ctenophores. Their delicate structure and relatively short life under laboratory conditions, together with their somewhat sporadic appearance within the reach of laboratories, have made investigation of their habits difficult. Mayer ('12) notes that "young *Beroë cucumis*" devours *Pleurobrachia* "with avidity." Bigelow ('15) observed the great impoverishment of the plankton on German Bank due to *Pleurobrachia pileus*, which "when it swarms seems to obliterate or devour almost everything else in the water." Kincaid ('15)

[5] Moore ('24) observed the beating of the paddle plates of *Mnemiopsis* at − 0.6° C. under laboratory conditions.

states that *Pleurobrachia* may be a serious enemy of the oyster in Washington waters through the large numbers of the larvæ of the latter which it consumes.

In a recent work, the most extensive of its kind with which I am familiar, Miss Lebour ('22, '23) describes the food of numerous plankton organisms. She lists *Pleurobrachia* and *Beroë* with *Sagitta* among the miscellaneous feeders of the plankton. Where these two ctenophores occur together *Beroë* may eat large numbers of *Pleurobrachia*, an observation which was also published by Mayer, however, in 1912. The chief food of *Beroë* was found by Miss Lebour to be small crustacea, although she quotes one observation of a *Beroë* full of diatoms, probably *Coscinodiscus*. *Pleurobrachia* was found to subsist mainly upon *Calanus*, crab zœa larvæ, *Sagitta*, other *Pleurobrachia*, *Syngnathus*, young plaice and plaice eggs. I have not had opportunity to determine the food of *Pleurobrachia*. The *Beroë* which were found September 11, 1924, were living chiefly upon *Mnemiopsis*.

The abundance and persistence of *Mnemiopsis* in Barnegat Bay in 1923 gave opportunity to study the food habits of this ctenophore during the seasonal changes of the plankton. A few preliminary examinations demonstrated that at summer temperatures the rate of digestion of food and the ejection of residue is so rapid as to make necessary the examination of the ctenophores immediately upon removal from the water. In most instances the stomodeum was emptied of all its contents in from 20–30 minutes after removal of the animals from their natural surroundings.

The great transparency of this organism makes it possible to identify with a high degree of accuracy the contents of the stomodeum without in any way disturbing the animal. In making the examinations the station launch was anchored in the desired spot in the open bay and the ctenophores were dipped up in a fingerbowl as needed and examined immediately under the binocular.

During the summer the food of *Mnemiopsis* was found to consist chiefly of larval molluscs, copepods and their nauplii, nannoplankton, and detritus, the relative amounts of these being to some extent correlated with their abundance in the plankton. In this connection I wish to lay emphasis upon a fact

which, so far as my knowledge goes, has not hitherto been stressed. The presence of nannoplankton and of organic debris within the ctenophores demonstrates that these animals make use of their ciliated canals for the transport of minute forms in much the same manner as does a bivalve mollusc. The possession of this feeding mechanism makes available to them the nannoplankton which is by far the greatest constituent of the total plankton, a constituent, moreover, which may be mainly unavailable to their larger cœlenterate allies. This may explain, in part, how such vast hordes of ctenophores can exist together for long periods of time (*cf.* Nelson, '22).

Table II. contains a summary of the food organisms found in *Mnemiopsis* during July.

To summarize the data in Table II.: of 65 *Mnemiopsis* examined during July, 75 per cent. had eaten bivalve larvæ, 50 per cent. contained crustacea, 15 per cent. held gastropod larvæ, while 6 per cent. contained detritus and nannoplankton. One specimen about 3 cm. long had eaten 126 early oyster larvæ.

On December 21, of 10 specimens examined 5 contained a total of 26 large *Calanus* and 1 gastropod larva. Examination of the ctenophores at this time and again on January 19, 1924, revealed a most interesting fact regarding digestion in *Mnemiopsis*. Copepods, as is well known, contain large oil globules, representing stored nutriment. In every ctenophore examined during the winter, oil globules derived from the copepods, were found to be deposited in thick rows beneath the paddle plates. Many minute oil globules were seen passing out of the anus, and one specimen was observed in the act of casting out through the mouth a thick rope of oil globules and detritus. Apparently but little of the oil obtained from the crustaceans used as food is metabolized by the ctenophores, at least at low temperatures. The storage of the oil in such large quantities beneath the paddle plates may serve an important function in decreasing the specific gravity of the body. This accumulation of oil at a time when also the density and the viscosity of the water are greatly increased through low temperature, renders the animal capable of suspension in the water with a minimum of activity of the paddle

plates. That the added factor of buoyancy has survival value during the late fall and winter can scarcely be doubted.

TABLE II.

Date.	Total Number Examined.	Oyster Larvæ.		Other Bivalve Larvæ.		Crustacea.		Gastropod Larvæ.		Detritus.		Miscellaneous.
		Larvæ.	*Mnemiopsis.*	Larvæ.	*Mnemiopsis.*	Copepods and *Nauplii.*	*Mnemiopsis.*	Larvæ.	*Mnemiopsis.*	Amount.	*Mnemiopsis.*	
July 12....	11	301	9	2	2	1	1	3	1			
July 13....	40	74	12	58	14	93	35	3	2	Much	2	1 roundworm 1 atax
July 18....	14	77	12			21	6	12	7	Much	2	1 prawn, 3 mm.
Total.....	65	452	33	60	16	115	42	18	10		4	

An interesting observation illustrates one limitation of *Mnemiopsis* in its ability to capture planktonts. The water was swarming with polychæte larvæ on December 21, but in spite of the fact that many of these larvæ were found entangled in mucus on the oral lobes and on other parts of the bodies of the ctenophores, in no case were any larvæ found within the stomodeum. The wealth of spines which bristle in all directions on the early larvæ of polychætes evidently render their final capture by *Mnemiopsis* difficult if not impossible.

That the food organisms found within the stomodeum of *Mnemiopsis* are actually digested is shown by the presence of numerous empty shells of bivalve and gastropod larvæ, crustacean carapaces, and other non-digestible remains which accumulate near the oral end of the stomodeum.

V.

THE DESTRUCTION OF BIVALVE LARVÆ BY *Mnemiopsis*.

Our records of the abundance of bivalve larvæ and the intensity of "set" in Barnegat Bay for the past 3 years show, after taking into account the effects of all known factors, that there is a close correlation between the abundance of *Mnemiopsis* and the

intensity of shipworm infestation and of oyster sets. In 1921 and in 1922 heavy sets of *Ostrea*, *Teredo*, and *Bankia* occurred. The oyster set in 1921, especially, was the heaviest seen for some years, as high as 1000 oyster spat attaching to one oyster shell (Nelson, '23A). The sudden and heavy outbreak of *Teredo navalis* at the same time (Nelson, '22) gave rise to fears that there might be enacted in eastern waters a repetition of the San Francisco Bay disaster (Kofoid et al., '21). It will be noted in Table I. that *Mnemiopsis* was absent or rare in Barnegat Bay and adjoining waters during 1921 and 1922.

The oyster set in Barnegat Bay in 1923 was a failure commercially. The best set that could be found at the close of the summer was one or two spat on every third oyster shell. The total season's catch on certain experimental shells was only 7 spat as against over 7000 in 1921.

Teredo navalis infested timbers in numbers as great as 100 per cubic inch of wood in 1921, while in 1923 at the same spot only 10 *Bankia* entered a test raft of 1,520 square inches surface. This very light infestation occurred in spite of the prevalence of slightly higher salinities obtaining in the region than were found in 1921. At only one locality, the jetty at the mouth of Barnegat Creek at the lower end of the Bay, could enough *Teredo* be found even for carrying on experiments with them. This jetty has been heavily attacked by borers for some years and was practically destroyed in 1921. Two infested piling were removed from this structure on July 25 and 5 were taken August 31, 1923. They were moored in a small land-locked creek close to the upper end of the bay, far from marine structures, and were used as a source of supply for study of the heterotrichous ciliate *Boveria teredinidi* Nelson, parasitic upon the gill filaments (Nelson, '23*B*). Throughout the summer and autumn until freezing occurred the borers grew and flourished, thus proving that no natural conditions present in the bay during this period were inimical to the existence of the adults.

The Director of the Committee on Marine Piling Investigations of the National Research Council, Col. Wm. G. Atwood, informed me in a letter of October 23, 1923, that "on the whole shipworm attack on test blocks was much lighter during 1923 than in 1922, although at one or two points it appears to have been as heavy

or heavier." Unfortunately for the purposes of this discussion there are no data as to the relative abundance of *Mnemiopsis* or of other plankton feeders in the several regions.

The relative reduction in numbers of oyster larvæ in 1921 and in 1923 is of interest. For example, on June 25, 1921, the average number of earliest straight hinge oyster larvæ per 100 liters of water, collected at 7 stations on Barnegat Bay, was 36,200. On the 27th, by which time the larvæ were entering on the early umbo stage, the average number of these larvæ was found to have been reduced to 8,700, representing a quite usual precentage of mortality. In 1923, on June 23, at the central collecting station near the middle of the bay where the early oyster larvæ have been first found in abundance for three successive years, there was obtained a total of 60,850 larvæ per 100 liters. Two days later the average of 8 stations showed but 54 of these larvæ remaining per 100 liters, the largest single catch at any one station being 200. So far as known there were present during 1923 no other factors aside from vast swarms of *Mnemiopsis* which were not also operating in 1921.

It is worth while to add a comparison between oyster sets found in Barnegat Bay by late August, 1923, with those at other oyster producing areas of New Jersey. In the Mullica River an excellent set of oysters one week old was found on August 30. At this date only an occasional *Mnemiopsis* was seen. Although no observations were made in this area earlier in the season there is no reason for believing that the ctenophores were more abundant during the preceding 3 weeks, during which time the oyster larvæ would have matured and set, than they were on the date of my observations. In Delaware Bay also the usual oyster set occurred. The waters of this area are so stormy and turbid near the shore that *Mnemiopsis* cannot thrive there, although the much hardier *Beroë* appears there in abundance in September, as already stated.

VI.

Discussion of Species.

To the student of the Ctenophora it is apparent that certain wide discrepancies occur between the account of the distribution of the three ctenophores discussed in this paper and the commonly

accepted ideas regarding the habitat of these species. All are usually considered as being strictly oceanic forms, and yet I have taken them in waters ranging from one half to less than a third the salinity of their supposed usual environment.

Mayer '12 holds that there are but two dominant species of *Beroë*, *B. cucumis* of cold waters and *B. ovata* of warm water. *B. forskali*, which was found at numerous stations by Bigelow, is believed by Mayer to be but a variety of *B. ovata*. Bigelow ('15) lists *B. cucumis* from 7 stations at some distance from the coast of New England. He does not record it off the New Jersey coast in July, nor does he mention *B. ovata*.

Mayer ('12) notes that the young of *B. cucumis* cannot be distinguished from those of *B. ovata*. In the adult the peripheral network of vessels arising from the meridional canals freely anastomoses in *B. ovata*, remaining distinct in *B. cucumis*.

Pleurobrachia brunnea, a species created by Mayer '12 to receive a ctenophore found off the New Jersey coast in October, 1904, is of somewhat doubtful position. Bigelow ('12) believed this to be so close to *Hormiphora spatulata* Chun, as to be identical with it. In a later publication ('15) he throws still further doubt on the validity of the species, believing it to be well within the limits of *P. pileus*, a ctenophore of wide distribution which he found in great numbers off the New Jersey coast in July 1913.

The characters used by Mayer to establish *P. brunnea* as distinct from *P. pileus* are: the continuation of the 8 meridional canals for a considerable distance downward beyond the ciliary combs; the yellow color of the stomodeum; and the knobs on the ends of the tentacles. It is distinguished from *Hormiphora spatulata* by the knob-like ends of the tentacles; and by the fact that its ciliated combs begin at a greater distance from the apex than in *H. spatulata*.

My own specimens agree in every particular with Mayer's description and figure of *P. brunnea*, the yellow color of the stomodeum and the knob-like ends of the tentacles being quite striking in the living specimens. The downward continuation of the meridional vessels below the ciliary combs is clearly evident in the preserved as well as in the living specimens. In considering Bigelow's belief that this form may lie within the limits of variation of typical *P. pileus* it is well to remember that

Bigelow obtained his specimens off the New Jersey coast in July, whereas Mayer and I found our ctenophores in October. The three distinguishing characters of *P. brunnea* as established by Mayer are clearly evident even to one who is but little familiar with the characteristics of this group. The chief question would seem to be whether this organism should be raised to specific rank, or be included as a variety of *P. pileus* or of *Hormiphora spatulata*. Its appearance in New Jersey coastal waters in October would seem to point to its being essentially a cool water form and hence closer to *P. pileus*.

The range of *Mnemiopsis leidyi* is given by Mayer as from the southern coast of New England south to the Carolinas. It is considered by Mayer to be a ctenophore of the pure sea water along the outer shores; its place being taken in the brackish water by its much smaller relative, *M. gardeni*. Bigelow ('15) agrees with this from his own findings. Nowhere in the accounts of either of these investigators do I find evidence that they made any careful examination of the estuaries of the New Jersey coast for there *M. leidyi* occurs in abundance for months at a time in water of as low as one-third the salinity of the sea or even below this. On one occasion I found it in upper Delaware Bay, just below Stony Point, in water of a specific gravity less than 1.005.

That I have not confused *M. leidyi* with *M. gardeni* will be evident from the following: (1) *M. gardeni* is described by Mayer as being 35–40 mm. in length when mature. I have found *Mnemiopsis* over 100 mm. long in Barnegat Bay. (2) The oral lobes of *M. gardeni* are very small, from one-fifth to one-sixth as long as the body. In all specimens of *Mnemiopsis* which I have seen, excepting only immature individuals not yet fully emerged into the Mnemiopsis stage, the oral lappets are much longer than this and they flare widely. (3) My specimens show, when large numbers are together, a decided pinkish hue, not the bluish color of *M. gardeni*. (4) The range for *M. gardeni* as given by Mayer is from Chesapeake Bay to Florida. (5) the striking power of adaptation of our species to low temperatures argues against its being the southern form. (6) I do not find in my specimens the small low discoidal warts on the oral lobes which according to Mayer are characteristic of *M. gardeni*.

VII.

The Distribution of Ctenophores as Effected by Salinity and Temperature.

Mayer ('14) has emphasized the superior temperature adjustment of marine animals of the arctic and temperate zones over individuals of the same or of related species in the tropics. *Mnemiopsis leidyi* in Barnegat Bay illustrates this superiority of adjustment in a striking manner. Owing to the typography and small tidal flow of Barnegat Bay, summer temperatures there may rise well above 26° C. During most of the summer of 1923 the water temperature varied between 24 and 25° C., with specific gravity over the region within 4 miles south of Seaside Park ranging from about 1.0110 to 1.0180.

Bigelow ('15) found *Mnemiopsis leidyi* in July in greatest abundance over the inner half of the continental shelf off the New Jersey coast, in water of a specific gravity between 1.0246 and 1.0252. It was not found in the very salt Gulf stream on the one hand nor where the specific gravity fell below 1.0244 at the mouth of Chesapeake Bay, on the other. The upper limit of temperature at which this ctenophore was found was 24.5° C., the lower about 15.5° C.

He gives the range of *Pleurobrachia pileus* as unbroken from Labrador at least to Pamlico Sound. He found it more generally distributed in the coast waters than any other cœlenterate, with local swarms south as well as north of Cape Cod. The warmest water in which it was found was 20.5° C., the coldest about 6° C. The range in specific gravity was from about 1.0241 to approximately 1.0267. Nearly all the specimens taken were from the deeper waters, whereas the majority of *Mnemiopsis* were found in the first fathom. The swarms of *Mnemiopsis* and *Pleurobrachia* were found to be mutually exclusive; never were these two forms taken side by side.

The distribution of *Pleurobrachia* in the deeper waters, together with the lower temperatures observed, indicate that *Pleurobrachia* is adjusted to colder water off the New Jersey coast than is *Mnemiopsis*. The high summer temperatures of the estuaries such as Barnegat Bay form a barrier which it, unlike *Mnemiopsis*, is unable to pass. In the extreme southern part of its range

Pleurobrachia is undoubtedly adjusted to temperatures equal to if not above those found during the summer in Barnegat Bay.

Pleurobrachia brunnea may be a species or subspecies which is delicately adjusted to a narrow range of temperature, since it is found in the inland waterways of New Jersey only during October, and it was taken in the ocean off Barnegat by Mayer during the same month. It is not stated at what depth these were obtained nor is the temperature given. As bearing on Bigelow's finding that *Pleurobrachia pileus* and *Mnemiopsis* were mutually exclusive, it will be remembered that in the swarms observed by me in the Mullica River *P. brunnea* and *M. leidyi* were taken together in large numbers at the surface in water dipped up with a fingerbowl.

In conclusion, consideration must be given to the part which salinity and temperature play in the sporadic appearance of the three ctenophores discussed here. A study of all data relating to rainfall, seasonal temperatures, severity of the preceding winter, and other factors, fails to show any correlation between these and the abundance of the ctenophores. The fact that one or two specimens of *Mnemiopsis* have been taken every year at Woods Hole since they ceased to be found there in abundance, and the presence of occasional specimens in Barnegat Bay even during "off" years, indicates that the factors controlling the appearance of the ctenophores probably lie mainly outside the several localities which are considered in this paper.

All ctenophores disappear from the shallow bays during the winter, the waters being repopulated the following summer from the sea. Should the region in which Bigelow found *Mnemiopsis* in such abundance in the Atlantic Ocean off the New Jersey coast be shifted even a mile or two eastward during some years, it would probably pass beyond the influence of the tidal ebb and flow through Barnegat Inlet and as a result few if any *Mnemiopsis* would be found in the Bay. Long continued easterly winds do bring to our shores forms which do not normally occur there but the effects of such winds and the currents produced thereby are doubtless only temporary.

No one knows the effects on the plankton of coastal waters which result from even slight shifts in the direction, and of changes in temperature or of salinity of the larger oceanic

currents. Until extensive oceanographic investigations are conducted along our coasts during every month of the year for a considerable period, we shall continue ignorant of most of the underlying factors which finally determine the abundance and distribution of many of the transient visitors to our coastal waters.

Summary.

The occurrence and distribution of the three ctenophores, *Mnemiopsis leidyi*, *Pleurobrachia brunnea*, and *Beroë ovata* in New Jersey inland coastal waters are considered as affected by factors of the environment.

Pleurobrachia was observed twice, *Beroë* during a few days for three seasons, *Mnemiopsis* was seen daily for months at a time.

Evidence is presented which indicates that all three of these forms are casual visitors to the inland coastal waterways of New Jersey. *Beroë* and *Pleurobrachia* are found here only after the heat of summer is past, whereas *Mnemiopsis* appears in late spring or early summer and flourishes until late autumn or winter.

The remarkable power of temperature adaptation of *Mnemiopsis* is shown by the fact that in individuals taken in the winter the swimming plates continue to beat until frozen fast in the ice.

The three ctenophores are shown to be able to withstand salinities in the bays which are considerably below those of the ocean lanes from which the animals came. The conclusions of Mayer and of Bigelow in this connection are discussed.

A study of the food habits of Mnemiopsis reveals the fact that large numbers of bivalve larvæ may be destroyed by them. A distinct correlation is shown between the abundance of *Mnemiopsis* and the intensity of "sets" of oysters and of marine borers.

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STUDIES ON MICROSPORIDIA PARASITIC IN MOSQUITOES.

V. Further Observations upon *Stempellia* (*Thelohania*) *magna* Kudo, Parasitic in *Culex pipiens* and *C. territans*.[1]

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Introduction.

In the autumn of 1919, I chanced to observe at Urbana, Illinois, six larvæ of *Culex pipiens* which were infected by a microsporidian and named it *Thelohania magna* (Kudo, '20). Since the prospect of obtaining additional material was small at that time, I ventured to summarize the results of my observations made upon "a single section preparation and a number of smears" of the host larvæ in a paper ('21).

In the following year, I found at Warren, Pennsylvania, some forty-three larvæ of *Culex territans* which harbored an apparently similar protozoön, whose effect upon the host mosquitoes was the subject of another paper ('22). This material from Pennsylvania was far more abundant than the previous collection, and enabled me to conduct an infection experiment. The observations in the temporary field laboratory and subsequent microscopical studies of a large number of preparations have brought

[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 254.

into light some phases of the development of the protozoön which were not recognized in the scant material of 1919 and which now lead me to place the microsporidian in the genus *Stempellia*.

The present paper deals with the findings thus made with special reference to the development of the microsporidian starting with an experimental infection and is intended to supplement the observations published before.

Material and Methods.

The host larvæ, *Culex territans*, were found breeding in an old boat filled with rain water on Conwango River at Warren, Pennsylvania. On two occasions, July 5 and 10, 1920, 290 larvæ were collected and examined microscopically, of which 43 were found to be infected by the microsporidian.

The material was studied in fresh as well as fixed and stained smears and in section preparations. For fixation, Schaudinn's mixture was mainly used; for staining, Heidenhain's iron hæmatoxylin or Giemsa's stain was used as in previous studies. A few Giemsa-stained preparations were decolorized and stained with Heidenhain's hæmatoxylin, although the reverse was not attempted, in order to compare the effects of staining by the two methods. It may be worth while to state here that methylene blue M.P. seems to be a suitable stain for the spores. On July 7, 1920, I subjected a number of spores to mechanical pressure on a slide, added one drop of a strong aqueous solution of methylene blue and sealed with a vaselined coverglass. An examination of this preparation in the summer of 1923 showed that the spore membrane had not shrunk and that both the polar capsule and the sporoplasm in the spores which escaped the pressure were most distinctly visible (Figs. 4 *j*, *k*).

Since Fontana was not carried on the trip, smears of pressed spores were either stained with Giemsa's stain or treated with a mixture of Lugol's solution and gum arabic. Some of the smears were kept air-dry, however, and stained later with Fontana.

The Infection Experiment.

In order to determine the changes which the spores of the microsporidian undergo in the digestive tract of a new host larva when taken into it with food and if possible, the way with which

the parasite becomes established in the new host animal, an experiment was conducted.

A number of larvæ of *Culex territans* which had been collected on July 10 and which were normal in appearance and behavior, were set aside in a glass jar. At two o'clock in the afternoon, three heavily infected larvæ, cut into small pieces, were given the larvæ, which fed willingly upon these fragments immediately after the latter were placed in the water. Some of the larvæ fed only for a few seconds, others for several minutes. The latter were removed one by one by means of a pipette as soon as they ceased to feed, into another jar which was partly filled with the rain water that had been standing by the laboratory free from mosquito larvæ. Twenty fed larvæ were thus obtained by three o'clock. I was sure that they had eaten certain portions of the infected material; in fact, all of them showed, upon microscopical examinations which followed in from six hours to four days, that they had devoured a large number of spores and sporulating stages of the parasite. The majority of the larvæ which did not feed long or at all, were found pupated on the following days.

From another lot twenty larvæ were selected as control animals by macroscopical inspection of the group and a low power microscopical examination of the individual larvæ.

On account of the comparatively simple organization of the alimentary canal and its connected organs of the mosquito larva and of the large dimensions of the protozoön, the microscopical examinations of the material involved in the experiment both in smears and in sections were carried out with greater ease and certainty than that which I had experienced in the case of the infection experiments of *Bombyx mori* with *Nosema bombycis* (Kudo, '16), where the conditions were reversed.

In the study of the preparations related to the experimental infection, Giemsa's stain seemed to be indispensable, since it brought out the spores, particularly the sporoplasms with their nuclei, of the microsporidian, sharply before the multitude of other microorganisms of animal as well as plant nature, which existed in the rain water and which found their way freely into the alimentary canal of the insects. The distinction between the latter and the emerged sporoplasms of the present microsporidian

was the matter which occupied a great deal of my time. It was found that this distinction could only be done with great difficulty in the preparations stained with Heidenhain's iron hæmatoxylin, while it was comparatively easily made out in the sections stained with Giemsa's mixture. With Giemsa, the nucleus of a sporoplasm whether within the spore membrane or without, took a peculiarly bright crimson color, which led me to distinguish it possibly as such from those of the other organisms present in the lumen of the host gut.

The scheme of examination of both the experiment and the control larvæ was as follows:[2]

Time after Feeding on Infected Material.	Observations in Smears.	Sections.
6 hours	The alimentary canal of two larvæ was extracted from the body and studied in fresh conditions; later fixed and stained.	Two larvæ were fixed *in toto*.
24 hours	Two larvæ observed in a way similar to the above.	Two larvæ were fixed *in toto*.
40 hours	Four larvæ observed in a way similar to the above.	
2 days	The entire body of two larvæ was studied in fresh smears; later fixed and stained.	Two larvæ were fixed *in toto*.
4 days		Four larvæ were fixed *in toto*.

In the fresh preparations of the alimentary canal of the larvæ which were examined six hours after feeding on the infected material, a large number of unchanged spores with normal appearances were observed. Spores with a comparatively large clear area at the rounded extremity were noted in numbers (Fig. 1, *a*); spores with a small mass projecting from the attenuated end were also noticed (Fig. 1, *a*). When stained, the spores showed a relatively large nucleus (Fig. 1, *b*).

In the larvæ which were examined 24 hours after feeding on the infected material, the mid-gut contained a large number of unchanged spores and empty spores; some of the latter showed extruded filaments. A number of spores exhibited an appearance shown in Fig. 1, *c* in which apparently the emergence of the contents was taking place. This particular spore sketched here was kept under observation for thirty minutes with an oil

[2] Due to unexpected early departure from the place, the experiment was unfortunately discontinued on July 14.

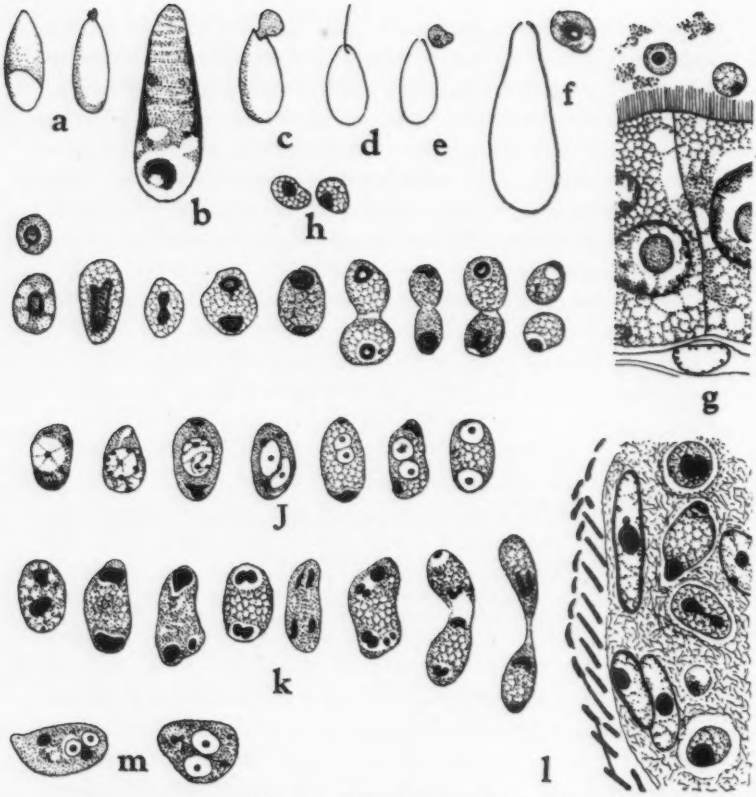

FIG. 1. Developmental stages of *Stempellia magna* observed in the host larvæ fed on infected material. *a*, *c–e*, × 1000; others, × 2300. *a*, fresh spores taken from the mid-gut of a larva six hours after feeding on the infected material. *b*, a stained spore observed in the same host individual (Giemsa). *c*, a fresh spore observed in the contents of the mid-gut of a larva 24 hours after feeding on the infected material. *d*, a spore found in the mid-gut contents of a larva, 40 hours after feeding on the infected material. *e*, a spore found in the same larva (empty spore membrane and the sporoplasm?). *f*, an empty spore and a uninucleated sporoplasm (?) from the mid-gut of a larva, 24 hours after feeding on the infected material (section; Heidenhain). *g*, two liberated sporoplasms (?) in the lumen of mid-gut of the same larva (Heidenhain). *h*, two young schizonts observed in the periintestinal fat body, 24 hours after feeding. *i*, *j*, *k*, schizonts in various stages of development and division as found in the fat-body surrounding the posterior part of the mid-gut and tracheæ in the larvæ examined 24 hours to four days after feeding on the infected material (sections; Heidenhain). *l*, five schizonts in the peritracheal adipose tissue near the mid-gut of a larva, 48 hours after feeding on the infected material (Heidenhain). *m*, the most advanced schizonts (sections; Heidenhain).

immersion objective, but the emergence did not advance any further. I cannot determine whether or not the form changed during this period, since the spore underwent Brown's movements which were further exaggerated by the turning of the finer adjustment of the microscope. Some spores extruded while under observation the filament from the attenuated extremity. At the moment of the extrusion of the filament, the spore showed a vigorous vibration which seemed to have been caused by the sudden unwinding and extrusion of the coiled filament.

In the larvæ which were examined 40 hours after feeding on the infected material, the mid-gut contained more empty spores than those with normal unchanged appearances. Some spores were seen with extruded filaments (Fig. 1, *d*). A number of small amœboid bodies were found in the vicinity of empty spores (Fig. 1, *e*). This amœboid body when first noticed was near three empty spores. It contained a small dark spot near the center. While under observation, I saw it undergoing a sluggish change of form. In the mid-gut of a larva which was fixed 24 hours after feeding on the infected material, uninucleated bodies were seen near empty spores (Fig. 1, *f*).

Since I was not able to completely follow the emergence of the sporoplasm, I cannot state positively that these uninucleated bodies were emerged sporoplasms. The fact that they appeared in a larger number as the empty spores increased and that they resembled closely in size and appearance to the intrasporal sporoplasms and to the youngest stages found in the periintestinal adipose tissue of the host larvæ, however, leads me to consider them as the sporoplasms which left the spores under the influence of the digestive fluid of the host. These amœbulæ are mostly found in the posterior portion of the mid-gut where a number of them were found between the peritrophic membrane and the gut epithelium (Fig. 1, *g*). How these amœbulæ reached the adipose tissue could not be determined, although search was made repeatedly.

The youngest schizonts found in the adipose tissue surrounding the mid-gut and the adjacent tracheæ are shown in Fig. 1, *h*, *i*. They were observed in the larvæ as early as 24 hours after feeding on the infected material. Round in form, it shows reticulated cytoplasm and a large and compact nucleus, the

chromatin granules appearing to accumulate in a peripheral layer (Fig. 1, *h*). The nucleus seems to undergo a direct division and forms two daughter nuclei (Fig. 1, *i*). The cytoplasmic body of the schizont grows at the same time, becomes constricted into two parts (Fig. 1, *i*) and finally divides into two uninucleated bodies. This division is most probably repeated in the early phases of the infection, the forms shown in Fig. 1, *l*, being mainly of this kind. The nucleus may sometimes show a karyosome in it; in such a nucleus the division seems to be initiated by that of the karyosome. Frequently the cytoplasm does not follow the nuclear division and the nuclei divide again (Fig. 1, *k*). This is usually followed by an elongation of the body and by a division into two portions in each of which two nuclei are to be found (Fig. 1, *k*).

Another type of division noticed was initiated by a great increase in the size of the nucleus (Fig. 1, *j*). The nucleus becomes vesicular and exhibits a distinct karyosome near its center from which achromatic threads radiate toward the periphery. The cytoplasm contains two or more deeply staining grains. The karyosome divides into two and a septum is formed between them while the deeply staining grains become condensed at the opposite ends. This nuclear division does not seem to be followed by immediate division of the cytoplasm.

As the result of these schizogonic divisions, stages such as shown in Fig. 1, *m*, are produced. These are the only forms which were observed even in the larvæ examined four days after feeding on the material. As the time after feeding elapsed, the number of the various stages of the schizonts present in the fat body increased, although in none of the larvæ stages of sporogony were observed. It may be of interest to note that in one of the larvæ fixed four days after the experiment was started, the follicular epithelium of the ovary on the left side of the host body was greatly replaced by these stages described here, although the young ova seemed to be free from the parasite.

Schizogony.

Young forms found in the adipose tissue of naturally infected larvæ are represented by Fig. 2, *a*. They are comparable with the late stages noted in the experimentally infected larvæ in that

both possess two nuclei characterized by a karyosome and deeply staining reticulated cytoplasm. The deeply staining grains in the schizonts (Fig. 1, *m*) already described, may become dispersed in the cytoplasm, though occasionally one sees similar bodies in the naturally infected forms (Fig. 2, *b*).

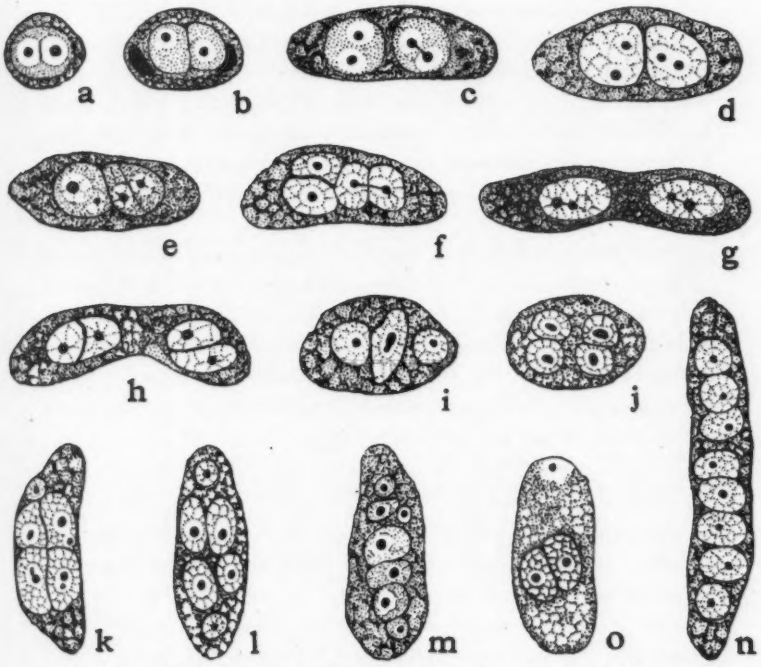

FIG. 2. Schizogonic stages of *Stempellia magna* observed in the sections of the host larvæ naturally infected. Heidenhain × 2300. *a–h*, further developmental stages of schizonts. *i–n*, stages which develop into multinucleate schizonts. *o*, the probable final stage of schizogonic multiplication.

The schizont grows large and the karyosome divides into two (Fig. 2, *c*, *d*) which is followed by the appearance of a membrane between them (Fig. 2, *e*, *f*, *h*). These changes result in forming two binucleated bodies (Fig. 2, *h*) which by further cytoplasmic division form two individuals such as shown in Fig. 2, *o*. The nuclei in the schizonts divide repeatedly without cytoplasmic constriction forming oblong schizonts possessing 3, 4, 5, 6, 7 and 8 nuclei (Fig. 2, *i–n*). These multinucleated forms seem to divide ultimately into binucleated forms (Fig. 2, *o*).

From the 1919 material I described both binary fission and multiple division of the schizonts which produced uninucleated forms; but a study of the preparation stained with Heidenhain's stain and also of the large number of 1920 preparations, leads me to think that the resulting form of the schizogonic divisions is a binucleate form such as are shown in Fig. 2, *o*.

As the two nuclei of the schizont come in a close contact, each karyosome buds off small chromatin granule which seems to be extruded into the cytoplasm later (Fig. 3, *a*). The nuclear membranes between the two nuclei disappear, while the two karyosomes become fused into one. The chromatin grains that were thrown out into the cytoplasm seem to divide further (Fig. 3, *b*).

This uninucleate body is the sporont and gives rise to spores through sporogonic development described below. The fusion of two "cousin" nuclei observed in *Thelohania legeri* (Kudo, '24) does not exist in the present species. Debaisieux ('19) describes a similar change in the schizonts of *Thelohania varians*. Other references on this point are omitted here, since it was brought up in detail in one of my recent papers (Kudo, '24).

Sporogony.

After growing somewhat in size the uninucleate sporont divides. Its karyosome divides into two which become separated by a nuclear wall and the two nuclei are formed (Fig. 3, *c*, *d*). The nuclei become separated from each other and locate themselves near the opposite extremities. A septum appears in the cytoplasm, and two sporoblasts are formed (Fig. 3, *d*). Each sporoblast develops into a spore.

Frequently the two daughter nuclei divide once more. The division begins with that of the karyosome, a strand remaining usually between the divided karyosomes. Thus a tetranucleated sporont is formed; the cytoplasm, as in the case of bisporoblastic sporont, divides into four sporoblasts (Fig. 3, *f*), each of which develops into a spore.

Less frequently a sporont nucleus divides three times, thus producing eight sporoblasts (Fig. 3, *g*) which later develop into eight spores.

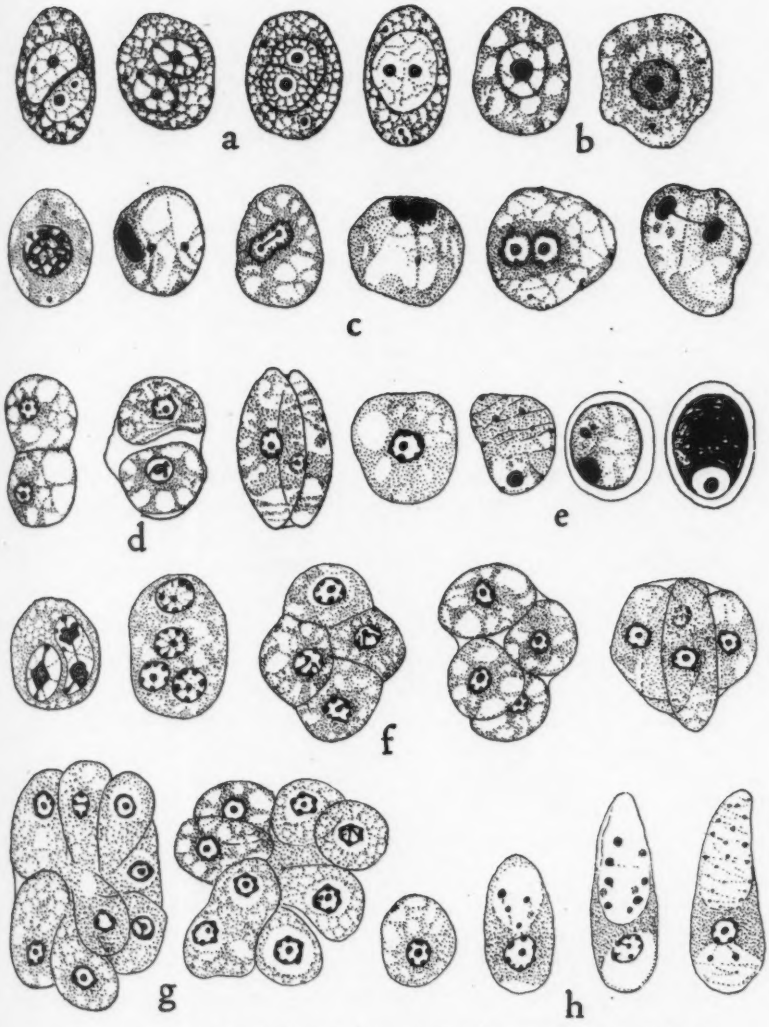

FIG. 3. Further developmental stages of *Stempellia magna* observed in the sections of the host larvæ naturally infected. Heidenhain. × 2300. *a*, four stages in the fusion of the two nuclei of the schizont which results in the formation of sporonts (*b*). *b*, sporonts. *c*, *d*, stages in disporoblastic sporogony. *e*, stages in monosporoblastic sporogony. *f*, stages in tetrasporoblastic sporogony. *g*, stages in octosporoblastic sporogony. *h*, stages in the development of a spore from a sporoblast.

Quite frequently the sporont when discharged into the body cavity of the host transforms into a sporoblast and later into a spore without any nuclear division stated above. This process probably is responsible for the production of abnormally large spores (Fig. 3, *e*).

The cytoplasm of the sporont is more vacuolated and less deeply stained than that of the schizont, of which I remarked before ('21). Schröder ('09) and Schuberg and Rodriguez ('15) mentioned a similar difference in the cytoplasm between the schizonts and sporonts of the Microsporidia they studied.

The sporoblast which varies greatly in size as the natural sequence of the difference in its production, is rounded or oval in form. It has a nucleus composed of peripheral chromatin grains and a karyosome. There is to be seen one or more chromatin grains near one end. The nucleus moves toward the other end of the sporoblast, while deeply staining granules appear in the clear space at the other extremity. These granules become smaller in size and larger in number as the filament is formed, which probably indicates that they are used for the formation of the polar filament. When nearly formed, the spores present the appearance shown in figure 3, *h*; the sporoplasm with one nucleus is near the round end and the coiled filament is present near the other extremity of the spore.

From the sporogony described above, it becomes obvious that the microsporidian cannot be placed in the genus *Thelohania* which is characterized by an octosporous sporont, as I held at first ('20, '21, '22), but should be placed in the genus Stempellia which Léger and Hesse ('10) established for *Stempellia mutabilis*.

The Spore

In fresh state, the fully formed spore is elongated pyriform, often bent slightly toward one side. In cross-section it is circular. One end which is ordinarily called as the posterior end, is rounded, while the other, the anterior end, is less rounded, though not attenuated. The spore is moderately refractive and presents somewhat varied aspects. In a large number of spores, there is to be seen an oval, cap-shaped or round area, through which a fine protoplasmic strand sometimes runs transversely (Fig. 4, *c*), while the other part is finely granulated and shows fine

irregular lines of coiled polar filament. In some spores, there is no clear space here noted and in others which apparently possess thin spore membrane, numerous transverse protoplasmic strands are to be seen (Fig. 4, *e*).

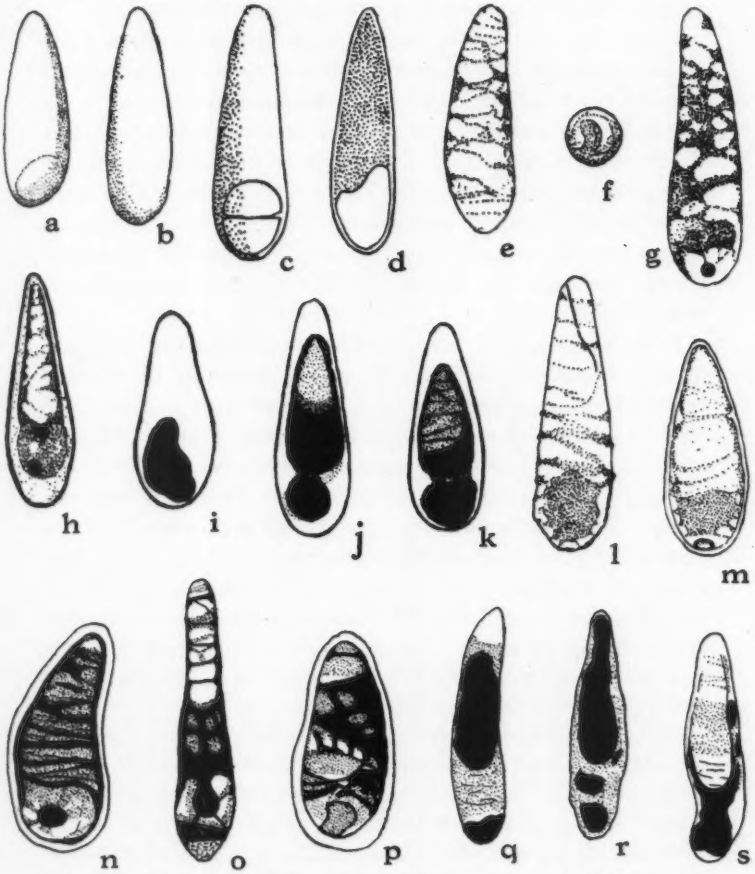

FIG. 4. Spores of *Stempellia magna*. *n–p* from sections; the rest from smears. × 2300. *a–e*, fresh spores. *f*, an end view of a fresh spore. *g*, *h*, spores stained with methylene blue M.P. and observed immediately afterwards. *i–k*, spores kept in methylene blue M.P. for three years under vaselined coverglass. *l*, *m*, spores pressed moderately and kept in Lugol and gum-arabic mixture for two days. *n–p*, abnormal spores, products of monosporous development (Heidenhain). *q*, *r*, normal spores (4 per cent. formol; Giemsa). *s*, normal spore (4 per cent. formol; Heidenhain).

The dimensions of the spore vary to a greater extent than those which I recorded from 1919 material. In fresh state, the spores measure 12.5 to 16.5 μ in length by 4 to 4.6 μ in largest breadth. Some abnormally large spores reach 25 μ by 10 μ, which are without doubt the products of monosporous sporogony.

When the spores are treated with methylene blue, there appears a deeply stained round body surrounded by less deeply staining cytoplasm which I hold as the sporoplasm, while in the remaining part, an irregular network becomes distinctly visible which is the coiled filament. In larger spores, the polar capsule does not seem to be present (Fig. 4, *g*); in the smaller ones, however, it is distinctly recognizable (Fig. 4, *h*, *j*, *k*). When the spores are kept in methylene blue, the polar capsule apparently shrinks and one sees the latter and a rounded sporoplasm in them (Fig. 4, *j*, *k*).

When the spore is subjected to mechanical pressure the filament becomes extruded. The average length of the filament is considerably greater than that obtained from 1919 material for which I gave 150 to 200 μ as the average length. Measurements of a larger number of spores with extruded filaments show that they average 350 to 400 μ in length. Except its base, the filament is of a uniform thickness which is less than one third of a micron in pressure-Fontana-preparations. When the pressed spores are mounted in a Lugol-gum arabic mixture and left for two days, the spore membrane, the sporoplasm and the extruded filaments take yellowish coloration. In such a preparation, the filament is considerably thicker, as a result of the swelling due to the medium used for the mounting (Fig. 5, *a*).

Contrary to some authors, there is no thickening at the distal end of the filament of the present form as was the case in all the other species of Microsporidia which I have studied up to the present (Fig. 5, *f*). Very rarely one sees a thick point at the extremity of the extruded filament (Fig. 5, *c*), an examination under a higher magnification shows, however, that here the filament became probably broken during the extrusion and the material which compose the filament became spread out as a result (Fig. 5, *e*).

Abnormal spores such as shown in Fig. 4, *n–s* are of frequent occurrence. Normal spores appear irregularly slender in form when fixed in smears with formol (Fig. 4, *q–s*).

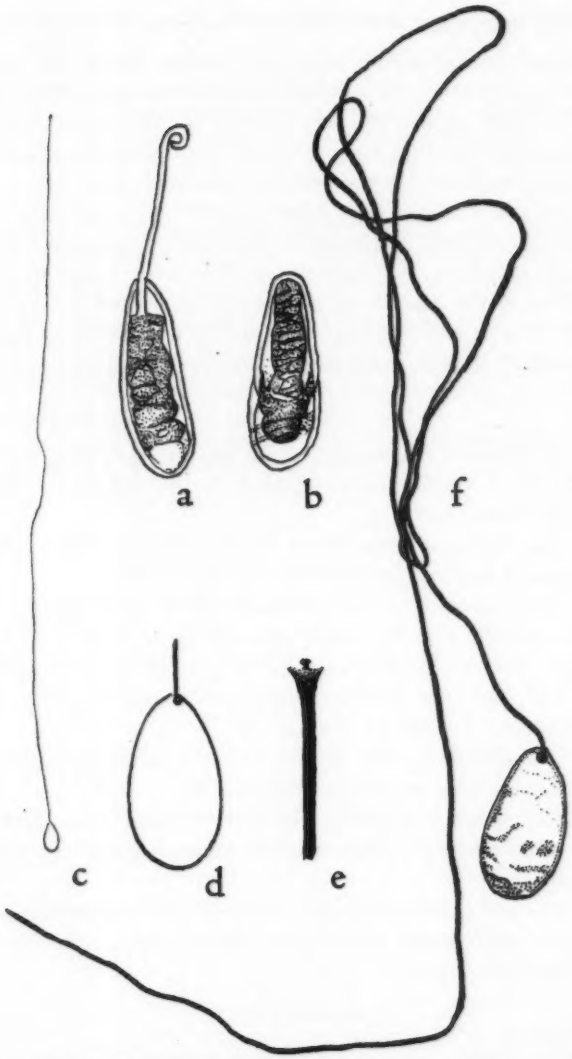

FIG. 5. Spores and polar filaments of *Stempellia magna*. Smears. *c*, × 360; *e*, × 3200; the rest, × 2300. *a*, *b*, spores pressed mechanically and kept in Lugol and gum-arabic mixture. *c*, a spore pressed mechanically and stained after Fontana. *d*, the same spore under higher magnification. *e*, the distal end of the filament shown in *c* highly magnified. *f*, a spore similarly treated, but stained with Giemsa.

The Relation between the Parasite and the Host.

I have remarked about this subject before (Kudo, '21, '22) and do not at present have any additional statement to add. So far I have failed to observe adult mosquitoes infected by the protozoön. But from what I have recently seen in the cases of infection of adult anopheline mosquitoes by *Thelohania legeri* and *Nosema anophelis* in Georgia, it is quite possible that the Culex larvæ lightly or moderately infected by *Stempellia magna* would be able to metamorphose into adults; on the other hand, when the infection of the host larvæ by the microsporidian is to such an extent as to show a typical symptom to unaided eyes, the host larva would die before completion of larval life.

Summary.

1. *Stempellia* (*Thelohania*) *magna* was found to be parasitic in the larvæ of *Culex pipiens* (Illinois, 1919) and of *C. territans* (Pennsylvania, 1920).

2. The infection experiment shows that the larvæ become infected by feeding upon the infected larval tissue.

3. The emergence of the sporoplasm of the spore taken into the gut lumen of a new host takes place in the posterior part of the mid-gut from 6 to 40 hours after feeding on the infected material.

4. The schizonts are first noticed in the adipose tissue of the mid-gut and of adjacent tracheæ.

5. The sporogony did not start in the larvæ examined four days after feeding on the infected material.

6. Schizogony is a binary fission of various types. The final form is binucleated. The two nuclei undergo autogamy, forming a sporont.

7. The sporont develops into ordinarily two, frequently one or four and rarely eight sporoblasts; these develop into two, one, four and eight spores.

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A MUTATION IN THE MOTH-LIKE FLY (*PSYCHODA ALTERNATA*) AND THE METHOD OF ITS TRANSMISSION.

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I. LIFE HISTORY AND GENERAL FEATURES OF THE NORMAL FLY.

The Moth-like Flies are inconspicuous insects about 2 mm. in length which breed in water, decaying vegetation and in manures. They have been little studied and consequently the life histories of only a few are completely known. In one or two instances they have been observed under laboratory conditions for two or three generations with the end in view of determining the lengths of the various stages of the life histories. There is no record, however, of their being carried under such conditions for more than a few generations.

During the past year the writer has reared successfully two species (*Psychoda alternata* and *Psychoda minuta*) and one of the two has proved itself to be an excellent subject for studies in heredity. A mutation has arisen in *Psychoda alternata* and the method of its transmission determined. A full account[1] of the life histories and of the general features of the two species mentioned has been offered in a previous paper so that no extended account will be necessary here. It seems necessary, however, to repeat the account in so far as it has to do with the mutation.

Eggs are laid upon moist manure by the females and within a few days they hatch producing active, feeding larvæ resembling those of midges. The larvæ possess pigment in two forms. Chitinous plates are present on the dorsal side of the last two or three segments (Fig. 1, *C.P.*) and a heavy chitinous sheath covers the head and the last segment (Figs. 1 and 2). These plates are light brown in the young larvæ but they grow darker with age. The other pigment is reddish brown in color and is found in the ocelli (Fig. 2, *Oc.*) and the Malpighian tubules of the larvæ (Fig. 1., *M.P.T.*).

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After eight days the larva becomes quiescent and pupates, spending two days in the pupal stage. The chitinous coverings become more extensive during this time and much darker, especially over the head and the thorax (Fig. 3). The Malpighian tubules become shorter and the reddish brown pigment in them becomes somewhat darker (Fig. 3, *M.P.T.*). During the pupal stage the compound eyes, each containing about seventy-five facets, develope. The same reddish brown pigment that occurs in the ocelli and in the Malpighian tubules also developes in the facets of the compound eyes. The chitinous walls between the facets become darkly colored but only after the reddish brown pigment of the facets is thoroughly established. During the formation of the compound eyes the ocelli degenerate.

The adult emerges as an extremely hairy insect with pigment present in three forms. The compound eyes (*CE.*, Figs. 4 and 5) and the Malpighian tubules (Fig. 4, *M.P.T.*) contain the reddish brown pigment first observed in the larval ocelli and Malpighian tubules. The chitinous covering of the body is colored with browns of various degrees of intensity and the hairs which cover the body and the wings range in color from white to brown and black. Especially on the wings the color changes of the hairs are so abrupt as to give a mottled appearance. Small patches of hairs, coarse and black, and very conspicuous are located at the ends of the wing veins.

The adult life lasts from two to nine days, depending upon the sex and upon the event of copulation. All normal adults are positively phototropic and negatively geotropic when they first emerge but become noticeably less sensitive to light and to gravity as they grow older. These two reactions play an important rôle in bringing the two sexes together for copulation. The adults are apparently sexually mature at the time of emerging and copulation takes place within a few hours. In copulation the male seizes the female with a pair of heavy terminal forceps.

II. Character of the Mutation.

The mutation varies from the normal fly in three phases of its structure and in its reaction to light. All these differences may be traced to a single scource, namely, the lack of the reddish brown pigment in all of those structures in which it ordinarily

occurs in the normal fly. This lack is first evident in the larva where the ocelli are colorless (Fig. 7, *Oc.*) and the cells of the Malpighian tubules are also devoid of color (Fig. 6). In the Malpighian tubules of the mutant fly there are granular bodies which in the normal fly bear the pigment but are here colorless. In the pupal stage the Malpighian tubules go through the same structural changes as in the normal fly but no pigment develops in the granular bodies. Neither do the ocelli of the mutant which up to this time have developed no pigment become colored. The compound eyes of the mutant which are formed at this stage are normal except that they, too, develope no pigment (Figs. 9 and 10). In the adult fly the reddish brown pigment is wholly lacking in the compound eyes (Fig. 10, *C.E.*) and in the Malpighian tubules (Fig. 10).

The other phases of coloration are not affected. The chitinous coloration in the larva, pupa and adult stages cannot be distinguished from that of the normal specimens. The pigment coloration in the chitinous walls of the compound eyes is entirely obscured in the normal fly by the heavier coloration in the facets but in the mutation, because of the lack of the reddish brown pigment in the facets, the coloration of the chitinous interspaces is very prominent (Figs. 9 and 10). The coloration of the hairs of the mutant is identical with that of the normal fly.

In their reactions to gravity the normal and mutant flies behave alike but there is a marked difference in their phototropic reactions. The normal brown-eyed specimens have a strong positive reaction to light while the white-eyed ones are practically indifferent to light of the same intensity. Strong light has a kinetic effect upon both normal and mutant flies.

III. Nature of the Determiner.

If a single determiner be postulated for the lack of the reddish brown pigment which appears in turn in the simple eyes of the larva, the Malpighian tubules of the larva, pupa and adult and the compound eyes of the pupa and adult it would seem to be sufficient to account for all phases of the mutation. The fact that the pigment occurs in all three phases or not at all would argue for such a determiner. If any case had arisen in which the reddish brown pigment occurred in one phase and not in another it would

necessitate the postulating of a determiner for pigment in each phase. This series of pigmentary phases greatly facilitates the work of identifying progeny since specimens may be positively identified as normal or mutation in larval, pupal or adult stages.

In some cases of inheritance the determiner for a character does not become expressive except at a single stage in the development of the animal. Usually it is in the adult stage that a determiner shows its influence. The determiner must necessarily be present in all stages up to the time of its expression but may be regarded as only a potential influence until the proper time is reached. It is interesting to note that in the present case the determiner exerts its influence at three separated stages in the animal's life history; in the formation of the ocelli, in the development of the Malpighian tubules and finally in the formation of the compound eyes. This would indicate that some general metabolic process was being interfered with whenever that process was operating. In this particular case, then, the determiner might be described as a regularly inherited "influence" upon the metabolic processes which are necessary to the elaboration of the reddish brown pigment.

IV. Method of Transmission of Non-pigmented Condition.

When it was first discovered that white-eyed mutants were appearing in a mass culture the culture was closely watched and the mutants were isolated as they appeared. From the number of specimens appearing it became evident that the generation in which they had been discovered was not the one in which they had arisen. It is quite probable that one of the specimens first seen and confined in the jar was a white-eyed individual which had copulated with one or more of the normal specimens confined with it.

The mass culture in which the mutants arose would in such a case be a mixture and without knowing the method of inheritance of the mutation it would be impossible to know whether either brown- or white-eyed specimens were homozygous for the character in question. Neither was it known whether the variation would be permanent and heritable or whether it would prove to be a fluctuating variation.

A. Preliminary Experiments.

Using the white-eyed and brown-eyed specimens with unknown genetic constitution from the mass culture preliminary crosses were made for the purpose of getting some information as to the heritability and possibly the method of inheritance of the mutation in case it proved to be heritable.

a. White-eyed by White-eyed Crosses.

Sixteen crosses of white-eyed by white-eyed specimens were made, the specimens being isolated at the time of their emergence to insure virgin stock. From all these crosses 731 individuals were obtained and all were white-eyed. The eyes and Malpighian tubules of all showed a total lack of pigmentation. The question as to the heritability of the mutation was thus answered while the completeness and the permanence of the mutation has subsequently been established by maintaining pure strains of white-eyed flies up to the present time.

b. Brown-eyed by Brown-eyed Crosses.

Brown-eyed flies were isolated from the mass culture in a very young stage and 163 crosses were made, 91 of which were successful. In some cases all of the resulting progeny were brown-eyed. For example, culture number 138 produced three brown-eyed males on the ninth day, fifty-three more on the tenth day and during the following four days twenty-two brown-eyed males and forty-seven brown-eyed females.

In a few cultures some white-eyed specimens appeared. All such cultures showed a comparatively large number of brown-eyed specimens but the ratio was variable. It was important, however, to have obtained white-eyed specimens from a brown-eyed by brown-eyed cross since it indicated that the brown-eyed condition was probably dominant and the white-eyed condition recessive.

c. Brown-eyed by White-eyed Crosses.

Twenty-eight matings were made using brown-eyed males and white-eyed females and twenty-four using brown-eyed females and white-eyed males. Both types of crosses produced the same result thus demonstrating that there was no sex linkage. Of these matings thirty-two were successful. In six lots of progeny

all specimens were brown-eyed. The remaining lots contained both brown-eyed and white-eyed progeny, the white-eyed varying in proportion from 27 to 58 per cent. of the total number in each lot.

d. Discussion of Results in Preliminary Crosses.

The results of the preliminary crosses indicated that the normal condition was behaving as a dominant and the mutation as a pure recessive. Assuming that the normal condition (*BB*) was dominant and the mutant condition (*bb*) was recessive six possible crosses could be made and the expectations in the progeny would be as follows:

Crosses.	Expected Ratios (Phenotypes).
bb × *bb*	100 % white-eyed
BB × *BB*	100 % brown-eyed
BB × *Bb*	100 % brown-eyed
Bb × *Bb*	75 % brown-eyed, 25 % white-eyed
BB × *bb*	100 % brown-eyed
Bb × *bb*	50 % brown-eyed, 50 % white-eyed

The full expectation was realized in the preliminary *bb* by *bb* crosses.

Since both pure brown-eyed (*BB*) and hybrids (*Bb*) were contained in the mass cultures from which the breeding stock was selected it would be expected that the brown-eyed by brown-eyed crosses might be either *BB* by *BB* or *BB* by *Bb* in which case all of the first filial generation would be brown-eyed; or they would be *Bb* by *Bb* in which case there would be expected a large proportion of brown-eyed individuals and a few white-eyed ones in the first filial generation. This expectation was also approximately realized in the preliminary brown-eyed by brown-eyed crosses in that a small proportion of white-eyed specimens were procured from such crosses.

In the brown-eyed by white-eyed crosses there were two possibilities, namely, that the brown-eyed specimens used were either pure (*BB*) or hybrids (*Bb*). If the brown-eyed parents were pure all the progeny of the first filial generation would be brown-eyed. If the brown-eyed parents were hybrids then 50 per cent. of the first filial generation would be white-eyed. This expectation was realized in some lots as far as the securing of

all brown-eyed progeny from brown-eyed by white-eyed crosses. However, the expectation of 50 per cent. white-eyed and 50 per cent. brown-eyed progeny was only approximately realized in the preliminary experiments.

B. Experiments with Pedigreed Stock.

The strains used in these experiments were selected from vigorous stock all the members of which had been recorded through several generations. The white-eyed strain was one which had been started soon after the discovery of the white-eyed mutation and one which had been inbred from the start. The brown-eyed stock was one which had produced only brown-eyed individuals for eight generations.

a. White-eyed by White-eyed Crosses.

All progeny secured from white-eyed pedigreed stock was white-eyed. About twenty-two thousand of these individuals have been recorded.

b. Brown-eyed by Brown-eyed Crosses.

Pure brown-eyed by pure brown-eyed crosses have produced only brown-eyed progeny. About sixteen thousand of such individuals have been recorded.

c. Brown-eyed by White-eyed Crosses.

Whenever pure brown-eyed were crossed with pure white-eyed individuals the resulting progeny were invariably brown-eyed. About twelve thousand such progeny have been recorded.

In the second filial generation of pure brown-eyed by pure white-eyed crosses 15,086 individuals were obtained from 167 successful crosses. Of this total number 11,338 were brown-eyed and 3,748 were white eyed.

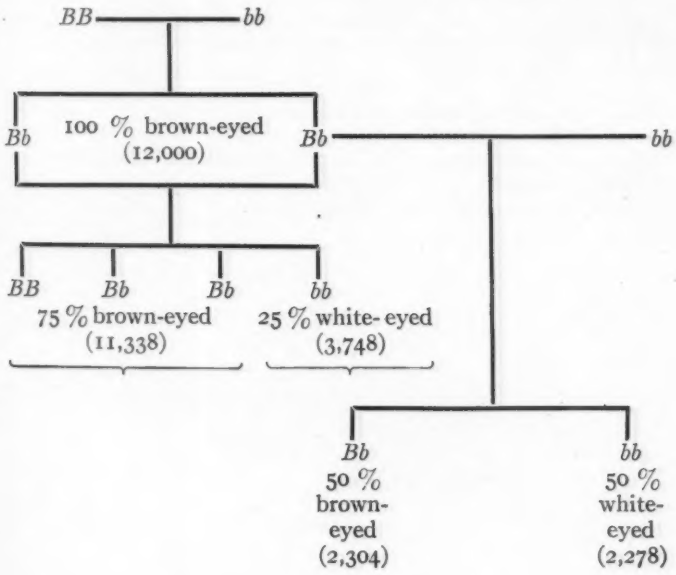
Back crossing the brown-eyed hybrid of the first filial generation with the pure white-eyed parental stock was carried out successfully thirty-seven times giving a total of 4,582 progeny of which 2,304 were brown-eyed and 2,278 were white eyed.

d. Discussion of Results of Experiments with Pedigreed Stock.

The results obtained verify the supposition that the condition represented by pigmented compound eyes, Malpighian tubules

and ocelli is dominant and the condition representing a lack of pigment is recessive. Since the results were uniformly independent of sex it follows that there is no sex linkage. The ratio expected in the second filial generation of a pure brown-eyed by pure white-eyed cross would be three brown-eyed to one white-eyed individual. The ratio realized (3.032 to 1) is near enough to the theoretical ratio to demonstrate that Mendels Law is operative. This is still further supported by the results from back crossing heterozygous brown-eyed stock with homozygous white-eyed stock. In terms of Mendel's Law it would be expected that one half of the progeny would be white-eyed and one half brown-eyed. A close approximation to this ratio was realized when out of a total of 4,582 individuals 2,304 were brown-eyed and 2,278 were white-eyed.

Following is a diagram illustrating the correlation of experimental results with expectations according to Mendel's Law for brown-eyed by white-eyed crosses. The number of individuals obtained in the experiments are shown in parentheses.



EXPLANATION OF FIGURES.

Note: All photographs were made from specimens killed and cleared in absolute alcohol and xylol and mounted in balsam. No stains were applied and none of the photographs have been retouched.

FIG. 1. Photomicrograph of the last four segments of a normal 10 mm. larva illustrating the Malpighian tubules (*M.P.T.*), the heavily chitinized terminal segment (*T.S.*) and the light dorsal chitinous plates (*C.P.*). × 24.

FIG. 2. Photomicrograph of the head of a normal larva illustrating the pigmented ocellus (*Oc.*). × 140.

FIG. 3. Photomicrograph of a normal pupa illustrating the Malpighian tubules (*M.P.T.*). The pigmented compound eyes are obscured by the dense chitin of the head and thorax. × 80.

FIG. 4. Photomicrograph of a normal adult male fly. In bringing the Malpighian tubules (*M.P.T.*) into sharp focus the compound eyes (*C.E.*) were somewhat blurred. × 30.

FIG. 5. Photomicrograph of the face of a normal adult fly illustrating the pigmented compound eyes (*C.E.*). The separate facets cannot be made out because of the uniform dense pigmentation. × 120.

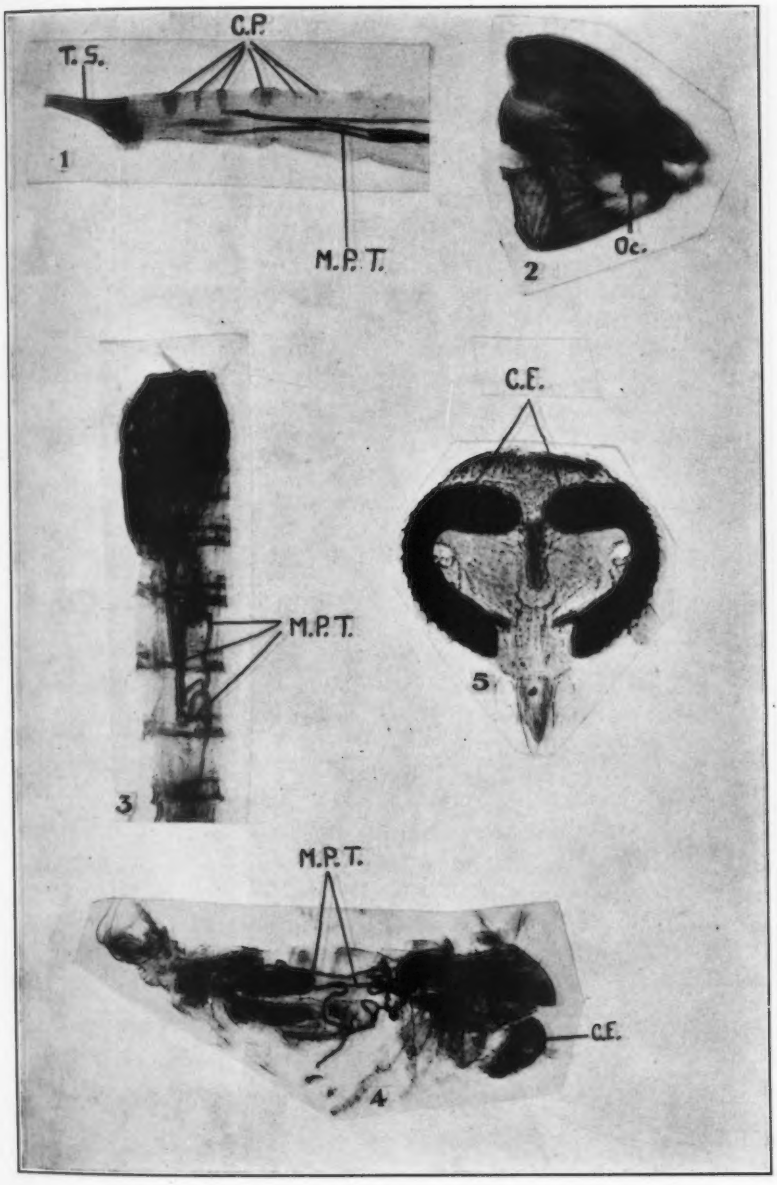


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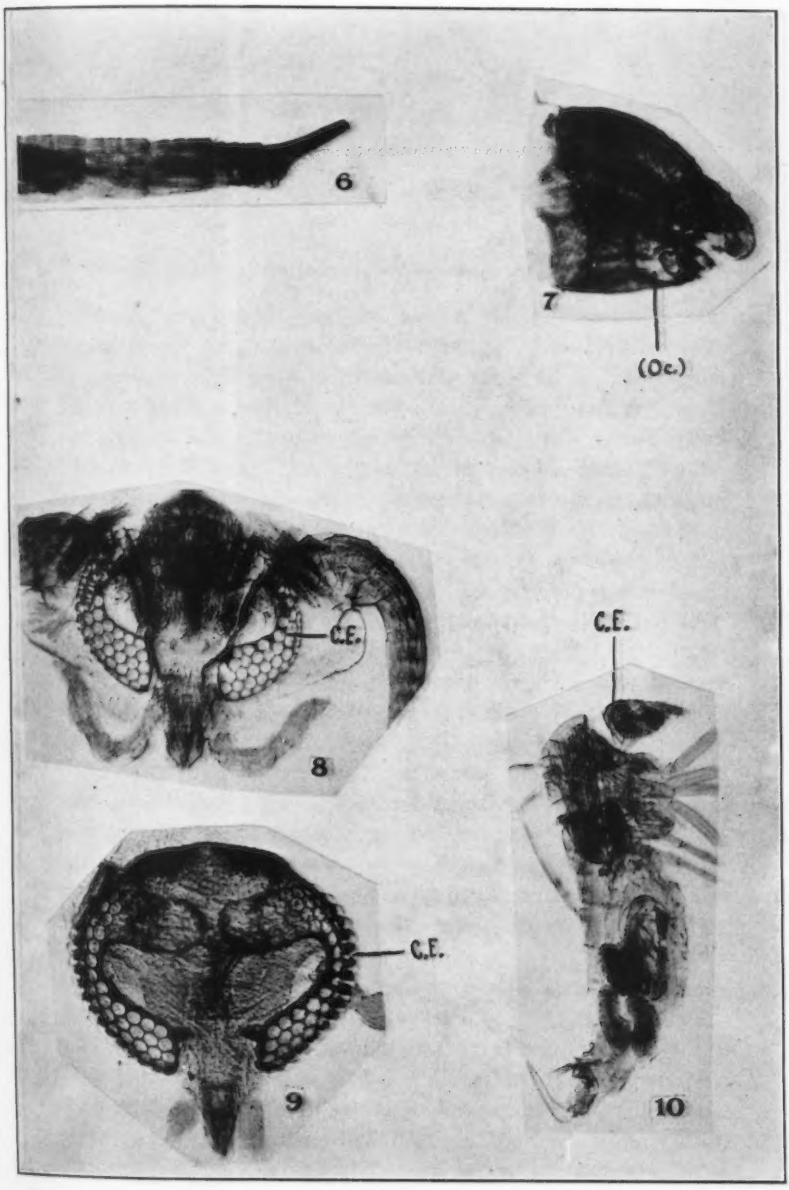
FIG. 6. Photomicrograph of the last four segments of a mutant larva. The Malpighian tubules are present but as they bear no pigment they do not appear in the photograph. × 21.

FIG. 7. Photomicrograph of the head of a mutant larva. The ocellus (*Oc.*) is not pigmented and so does not show in the photograph. × 140.

FIG. 8. Photomicrograph of the face of a mutant pupa illustrating the compound eyes (*C.E.*) which contain no pigment. × 100.

FIG. 9. Photomicrograph of the face of a mutant adult showing the compound eyes (*C.E.*). The margins and the interspaces have a yellowish-brown color that is characteristic of the heavily chitinized parts of the body but there is none of the dark reddish brown pigment in the facets that is found in the facets of the normal fly. × 132.

FIG. 10. Photomicrograph of an adult mutant fly showing the absence of pigment in the compound eyes (*C.E.*) and in the Malpighian tubules. × 30.



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REVERSAL OF FUNCTION IN A SPECIES OF *OLIGARCES*.

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There occur in *Oligarces* sp. two methods of reproduction: pædogenesis and adult sexual reproduction. These two methods are accompanied by a corresponding dimorphism among the larvæ of the species. Thus there are (1) pædogenetic larvæ and (2) pupa-larvæ. These two forms are functionally and morphologically different. The former larvæ, as the name implies, reproduce by pædogenesis, while the latter metamorphose into pupæ, which in turn become adults. Among the morphological differences existing between these two larval forms, three are of great value in distinguishing pupa-larvæ from pædogenetic larvæ, and vice-versa, both in the laboratory and in nature. These are (Harris '23):

"1. In newly born living pupa-larvæ the imaginal discs are visible. These occur laterally in the third, fourth and fifth segments. They are not present in pædogenetic forms.

"2. The spathula sternalis, a structure typical of Cecidomyid larvæ, occurs ventrally in the third segment of the pupa-larvæ of *Miastor*. It is not visible in newly born living pupa-larvæ, but after four or five days becomes clearly visible, due to a yellowish coloration which later changes to orange and dark brown. The shape of this structure differs with various species. Its function is not understood.

"3. The eyes of pupa-larvæ do not touch as in pædogenetic forms but are usually clearly separated."

These morphological differences correspond to those which Springer ('17) noted in *Miastor metraloas*. They have been confirmed in that species by the writer, and obtain in all species of *Oligarces* and *Miastor* which I have observed. Though many hundreds of pupa-larvæ, and thousands of pædogenetic larvæ

have been examined, none have been noted, either in nature or in the laboratory, in which the morphological appearance of the two larval forms did not correlate perfectly with the function of the larva, under normal conditions. Thus the writer and other observers have used the terms pupa-larvæ, and pædogenetic larvæ as definite terms (Springer '17, Harris '23, '24), based upon visible morphological differences, corresponding absolutely to functional differences. Pædogenetic larvæ consistently give rise, by pædogenesis, to other larvæ; whereas pupa-larvæ never give rise directly to other larvæ, under normal conditions, but continue their development by metamorphosis.

Recently I have indicated, as the result of a series of experiments, that pupa-larvæ may be obtained at will in the laboratory by crowding in the previous generation (Harris, '23, '24). During these studies, counts were made of pupa-larvæ which had arisen from crowded cultures of pædogenetic larvæ. It was found necessary, in order to make accurate counts to remove the pupa-larvæ from the original culture to a fresh culture, at the time of counting. This procedure removed the possibility of counting the same larva twice, and, at the same time, permitted counts to be made as pupa-larvæ arose in the culture; thereby making the larvæ available for other experiments.

Upon examining the abdominal contents of pupa-larvæ which had been transferred to fresh cultures, I observed in one pupa-larvæ not only eggs but young embryos. Both the eggs and young embryos were clearly distinguishable in the method employed.

Pupa-larvæ were placed in Ringer's solution on a slide and the anterior portion of the pupa-larvæ was removed with dissecting needles. The contents of the abdominal cavity were then forced out through the opening thus made; the larval skin was removed and the material was stained with a modified aceto-carmine preparation.[1]

The pupa-larvæ, already mentioned, appeared originally in culture M 187, which had previously been subjected to intense crowding. It, with other pupa-larvæ, was transferred to a fresh culture, MP 142, on December 30, 1923. When examined, as

[1] Acknowledgment is made for this staining method to Dr. Belling, of the Carnegie Institution of Washington, who has employed it with success in cytological work on *Datura*.

previously stated, 7 days later, the larva was found to contain 4 young embryos and 10 eggs. In the same culture another pupa-larva was observed with two large embryos, while other pupa-larvæ were observed containing varying numbers of embryos, though in no case more than four. On January 8, young larvæ escaped from several mothers, which had all the morphological characteristics of pupa-larvæ. These young, 33 in number, were typical pædogenetic larvæ, and later produced young pædogenetically in their turn. Of the total of 40 pupa-larvæ (which were transferred from culture MP 142), 27 metamorphosed into pupæ, and these in turn into adult flies, while 13, showing a reversal of function, did not metamorphose but gave rise to young in a typically pædogenetic fashion.

Since I had already observed that pupa-larvæ could be produced at will in the laboratory from a pædogenetic strain by crowding, it seemed probable that the apparent reversal of function was called forth by a reversal of the external conditions surrounding the pupa-larvæ. Thus the transference of a pupa-larvæ to a fresh culture immediately removed them from the influence of crowding, thus reversing the external conditions to which they had been subjected.

The fact that some of the pupa-larvæ seemed to respond to the treatment, while others did not, suggested the possibility that the age of the larvæ, when transferred, and by consequence the stage of development of the gonads and eggs, determined whether or not a reversal of function would occur.

(It may be said that the material upon which these observations were made is a female producing strain (Harris, 1924). The question then probably resolves itself into whether or not the eggs have reached a stage at which parthenogenetic reproduction is no longer possible.)

In order to ascertain if the apparent reversal of function were due (1) to the release from crowding, and from the consequent presence of an unusually large amount of the by-products of metabolism; or if it were dependent upon (2) the age of the pupa-larvæ when released from crowding, another series of experiments was carried on, using the same stock in the second subsequent generation. In this generation pupa-larvæ arose, as a result of crowding during embryonic developement, in culture M 222,

M 223, and M 224. These pupa-larvæ were transferred to fresh cultures, made of the same stock culture medium, and maintained under conditions as similar as possible to those of the control cultures. On this occasion, however, pupa-larvæ were separated into two age-groups.

It will be remembered that the spathula sternalis, a structure characteristic of pupa-larvæ in this material, is unpigmented when the larvæ are born, but becomes pigmented later, until, about three or four days after the pupa-larvæ is born, the spathula sternalis is dark brown in color. Thus the presence or absence of pigment in the spathula sternalis offers a rough index of age. This index was employed. Pupa-larvæ in which the spathula sternalis was unpigmented were placed in one series of fresh cultures, those in which pigmentation was clear were placed in another series. Four such cultures were formed, MPW 190, MPW 192, MPWO 191 and MPWO 193. The even numbered cultures contained only pupa-larvæ in which the spathula sternalis was pigmented; while in the odd numbered cultures none of the spathulæ sternalis was pigmented at the time of transfer. All larvæ were, from a morphological standpoint, typical. 16 pupa-larvæ with spathula sternalis pigmented were transferred to culture MPW 190; and 22 to culture MPW 192. Of the former, 7 metamorphosed into pupæ, and later into adults, while 9 produced young pædogenetically. In culture MPW 192, of the 22 pupa-larvæ present, 13 metamorphosed into pupæ and later into adults, while 9 gave rise to young pædogenetically. It is significant that in the cultures made from younger pupa-larvæ, as evidenced by the absence of pigmentation in the spathula sternalis when the larvæ were transferred, of the 15 pupa-larvæ in culture MPWO 191 only 1 metamorphosed into a pupa, and later into an adult, while 14 produced young pædogenetically. In culture MPWO 193, of the 8 pupa-larvæ present, none continued their development in the manner characteristic of pupa-larvæ, but all showed a reversal of function, giving rise to young in the manner of typical pædogenetic larvæ, though in most cases the number of young born was less than that of pædogenetic mothers from the same stock. All the young which were produced by the pupa-larvæ-mothers were, morphologically and functionally, typical pædogenetic larvæ.

TABLE I.

REVERSAL OF FUNCTION OF PUPA-LARVÆ FOLLOWING THE RELEASE OF YOUNG PUPA-LARVÆ FROM CROWDING.

Origin.	Transferred to Culture.	No. Showing Normal Function.	No. Showing Reversal of Function.	Total no. Pupa-larvæ.	Remarks.
M 187.....	MP 142	27	13	40	Age not considered.
M 222, 3 and 4 .	MPW 190	7	9	16	Spathula sternalis pigmented.
M 222, 3 and 4..	MPW 192	13	9	22	Spathula sternalis pigmented.
M 222, 3 and 4..	MPWO 191	1	14	15	Spathula sternalis unpigmented.
M 222, 3 and 4..	MPWO 193	0	8	8	Spathula sternalis unpigmented.

Culture M 187 was made up of larvæ of the F 10 generation of a strain maintained in the laboratory, cultures M 222, M 223, and M 224 represent the F 12 generation. This strain was subjected to crowding with the consequent production of pupa-larvæ in five of the ten generations. In no cases in which pupa-larvæ were not released from crowding did reversal of function occur.

In view of the results of this study the conclusion seems to be well founded that a reversal of the normal function of pupa-larvæ to that of pædogenetic larvæ can be obtained at will in the laboratory by reversing the conditions under which pupa-larvæ arose. This reversal has been obtained in the present instance by releasing pupa-larvæ from crowding.

Newly born and very young pupa-larvæ are more likely to give evidence of the influence of changed external conditions than are older pupa-larvæ. This probably indicates that a change, possibly reduction division, occurs in the eggs of the pupa-larvæ when the larva is still young. Before this change has occurred the eggs are still capable of pædogenetic development if external conditions are suitable. The progressive development of embryos within such a pupa-larva probably inhibits the normal

function of the larva to an extent that, instead of realizing its normal function, the larva, though originally a pupa-larva, functions as a pædogenetic larva. If the change has been accomplished the eggs will not develop pædogenetically, and the pupa-larva continues its existence in accomplishing its normal life-history.

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